

Rothschild May Be a Trojan Horse

SINCE the British government's endorsement of the customer-contractor principle for the conduct of research in its introduction to the green paper *A Framework for Government Research and Development*, there has been an uncanny silence about its intentions. In many ways, of course, this is laudably consistent with its declared intention of allowing time for public discussion of the important innovations which Lord Rothschild's contribution recommends. Behind the scenes, however, the government departments concerned in the reorganization have been sufficiently active to spread alarm among the research councils and to raise again the fear that in the continual battle for spending money, the Civil Service will regard the Rothschild report as an excuse for getting its hands on an even larger share of the cake than Rothschild recommended and that it will do so without proper regard for Lord Rothschild's own recommendation that the government departments should first of all be intellectually equipped to behave as intelligent customers.

In the scrabbling for budgets and for the influence which money brings, there is even a danger that the departments may go beyond the fields of research dealt with in the Rothschild report. There is, for example, talk that the Department of Trade and Industry has its eye on the funds at present spent by the Science Research Council on engineering research in the universities. The error here is a mistaken appreciation of the function of this expenditure, which is intended not as a direct contribution to industrial innovation but rather as a means of increasing the amount and improving the quality of basic engineering research in Britain, with the improvement of the status of engineering research which that would bring.

The difficulty of making the government departments into intelligent customers is likely to be seriously underestimated, especially by the departments. Lord Rothschild says that many of his recommendations entail "changes in attitude, orientation and procedure which [it] will take time to accept, let alone digest". The public debate which is now launched would be enormously helped by some sign that the government departments appreciate the enormity of the changes which will be required of them. No purpose would be served if they were simply to take over from the research councils the administration of parts of the existing budgets.

If the customer-contractor principle means anything at all, it requires that the management of research should be guided by objectives which are explicitly defined in advance. It will not be good enough to say let there be research on water resources, heart disease or ship design. The departments concerned would have to say what practical benefits they hope for. It cannot be too fiercely emphasized that this entails a radical departure from the present philosophy of research as at present carried out by the research councils. There, as Sir Frederick Dainton argues, fields of strategic research are chosen in the light of expectations that practical benefits of some kind will

mature, but the research councils do not pretend to specify these in advance. If in some fields, the Rothschild principle could be made to work, no doubt both the councils and the government would benefit, but there is nothing to be said for asking the departments to manage strategic research in the sense in which the research councils use the term. There is also a constitutional point at issue if government departments set out to manage scientific research by specifying objectives, they will also have to recognize the need to argue their choice of objectives not merely to the research councils which will be the contractors but also to the electorate, for research objectives will be political issues. In the broadest sense, that could also be a welcome development, yet few people will at present be confident of the capacity of the government as a whole to make intelligent public noises about its intentions in technical matters.

Baby with the Bath Water

BRITISH industry has given a warm welcome to the plans of the Department of the Environment for the reorganization of water and sewage services. The nub of the government's proposals, largely in line with the recommendations of the Central Advisory Water Committee last April, is that the complex of 29 river authorities, 1,200 sewage authorities and the British Waterways Board should be replaced by ten Regional Water Authorities, the boundaries of which have not yet been precisely defined. The only fly in the ointment is the proposal to replace the Water Resources Board in April 1974 by a part-time Water Council dominated by the ten chairmen of the Regional Water Authorities. This flies in the face of the recommendations of the Water Resources Board itself, the Central Advisory Water Committee and also Mrs Lena Jeger's working party on sewage, whose report was published last year. The danger, of course, is that the management of water resources in Britain will be deprived of the long-term planning and research which an independent central committee would have been able to provide.

The case for the abolition of the Water Resources Board, as outlined by the government, is that under the new arrangements, the regional authorities will be large and powerful enough to stand on their own feet, except for departmental supervision. Under the new arrangements, it will be for the regional authorities to arrange for transfers of water from one to another and also to carry out research. The difficulty is that even with the simplified structure now proposed, some of the water schemes which are in prospect are large enough to be of national as well as regional importance. The estuary schemes at Morecambe Bay and the River Dee, for example, are of a scale which is certain to override the interests of individual authorities,



especially when, at the beginning, these are struggling to cooperate with each other.

Research and development may also suffer under the new arrangements. Who, for example, will ensure that the new authorities will not needlessly duplicate each other's work? Will there be a rash of schemes for ground water development when carefully selected pilot studies would be better? Will there be ten pilot desalination plants when a smaller number would be sufficient? And who, under the new arrangements, will plan and execute the long-term schemes which may easily be larger in scale than the vision of the regional authorities, however large, can encompass? Much, of course, will depend on the balance of power between the regional authorities and the new Water Council, which may yet have more bite than at present appears likely. But the danger is that if the government does not get the balance right, there will be a return to the position before the Water Resources Act of 1963, and the balkanization of British water resources.

Time for Change in Spain

EVER since the Second World War, Spain has been conspicuous among the nations of Western Europe for its backwardness in scientific research and development. The OECD has now published the thirteenth of its Reviews of National Science Policy, *Spain* (£0.85), which is for one thing a more explicit criticism of the machinery used by a member government for regulating domestic science policy than the OECD has ventured so far and also, by all accounts, something of a landmark in Spanish affairs.

The diagnosis which the OECD's review team has presented goes a long way to explain why Spain has for a long time been too remotely connected with the rest of the scientific community. For one thing, the amounts of money spent on research and development are too small, whatever yardstick is used for measurement. In 1967, the government and industry between them appear to have spent 3,837 million pesetas (£22.7 million) on research and development of all kinds. This amounts to 0.27 per cent of the GNP of Spain in 1967, a somewhat flattering statistic when it is remembered that the per capita GNP was roughly a third of that of Belgium, for example. Throughout the 1960s, industry and the government in Spain seem to have been contributing almost equally to the total cost of research and development, but almost all of what the government spent was channelled through its own research centres, with the result that only three per cent of all intramural expenditure was in university research. How, it will be asked, can a modern state expect to run a university system with just over £600,000 for research? In the circumstances, it is clearly something of a miracle that Spanish universities have been able to win a high reputation for themselves.

The OECD review has also drawn attention to serious anomalies in the way in which the government's own expenditure has been channelled through the government research institutes. In spite of the importance of agriculture in Spain, expenditure on agricultural research in 1967 amounted to only 375 million pesetas, 17.4 per cent of all government expenditure on research and development. Moreover, between 1967 and 1969, the budget of

the Alonso de Herrera Foundation, which supports the work of several important agricultural research centres, declined by almost a fifth. On the face of things, it seems as if the Ministry of Agriculture had had early wind of the Rothschild proposals, for direct expenditure by the ministry was increasing. Of the small sums available for research and development, 23 per cent were devoted in 1967 to nuclear energy, and although the OECD review says that this may be entirely proper if indeed Spain is to generate a great deal of its electricity from nuclear power stations later in this decade and in the 1980s, this allocation of priorities must seem to outsiders to be misplaced.

The OECD review committee has several suggestions for reform, many of which were by all accounts discussed at a confrontation with Spanish officials in Madrid in the summer of 1970. Briefly, it urges that the government of Spain must choose between "the pattern of the past" and radical departures from it. It argues that research and development will make valuable contributions to the balance of payments, both in agriculture and in industry—the Ebro research centre has done well in agriculture, but elsewhere progress has been disappointing. The report is tart in its strictures on the government's neglect of university research, and pleads for the involvement of the universities in long-term planning—it could with advantage have said more about the need to improve the status of university research and to increase its scale. What would this cost? In round numbers, the OECD asked for a doubling of government expenditure on research and development as a first step. Luckily, there is now at least a chance that some part of this message will be heeded.

100 Years Ago



The Rigidity of the Earth

ALTHOUGH, as he truly says, Sir W. Thomson's arguments for the rigidity of the earth have never been attacked, yet they have undoubtedly been too long ignored; and it is gratifying to see them asserted by their author in *NATURE*. Allow me, however, to remark on one sentence near the end of his quotation from the "Natural Philosophy," where Mr. Hopkins's observation is given, that the distribution of fluid matter within the earth is "probably quite local." Unless I am mistaken, Mr. Hopkins's opinion was, that its distribution is, as one might say, fortuitous. But, as I have elsewhere observed, the trains of volcanoes which accompany many of the great lines of elevation for enormous distances render the motion of such local distribution of fluid matter highly improbable, unless it be admitted that its presence is due to mountain elevations as a cause. I have suggested that this fluidity may arise from a diminished pressure beneath mountain ranges, owing to their mass being partly supported by the lateral thrust which has upraised them—a supposition which Mr. Scrope had already applied to account for an increased fluidity in the heated rock underlying a volcanic vent, when from any cause the pressure became less.

If any of your correspondents can propose another explanation of this remarkable coincidence comparable with the supposition of a rigid globe, it would be interesting to know it.

Hartton, Cambridge

O. FISHER

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OLD WORLD

ENVIRONMENT

The Third Coming

LAST week Dr Paul Ehrlich addressed the Conservation Society as its new president. Two thousand five hundred people packed Westminster Central Hall to hear him speak on his third visit to Britain. His subject was "The Population, Resources, Environment Crisis—Where Do We Stand Now?"

He began with a brief historical survey, explaining that the world's population has been doubling faster and faster and is now doubling once every 35 years. This, he said, was primarily the result of a decline in death rates. He warned of the effects of rapid population growth, comparing mankind with fruitflies, which land on a banana and breed at an alarming rate until their food supply is exhausted, when there is a crash in the population and most of the flies die. A couple of pregnant flies fly off, however, and the cycle starts again on another banana. And the difference between man and the fruitflies, said Ehrlich, is simply that man has only one banana.

Today, he said, between three and seven times the population that can be sustained at a reasonable level is being supported in the world, but only because capital resources are being burnt up at an extreme rate.

Turning from the world's population to its resources he said that between 10 and 20 million of the 60 million people who die each year die from starvation. If all the food in the world was divided equally among the population there would be just enough calories per person and not enough protein—we would all be undernourished. It is easy to meet the world's calorie demands, but when it comes to high quality protein the world situation is desperate. And the fisheries will not provide the answer. Today 60 million tonnes are harvested annually from the sea. Conservative estimates say that the highest possible sustained yield will be 100 million tonnes. If the world population doubles in 35 years, we will be 20 million tonnes over the supply limit in 35 years. If the optimistic predictions are right, the sea could yield 200 million tonnes a year, in which case demand will still exceed supply in 70 years' time.

Some steps, however, are being taken to counteract the problem. The green revolution is helping to increase yields in underdeveloped countries. But this is only buying time. Possibly the damage it does will eventually outweigh the benefits.

Dr Ehrlich argued that even if the world's population growth were

stopped by the year 2000—which is more optimistic than anybody's prediction today—there will still be 1,300 million Indians, the population of the underdeveloped countries will still be 2.5 times its present level and the demand on the world's resources will not fall off until well past the middle of the next century.

More energy goes into growing one pound of wheat than can be gained from eating it, he warned. And even if we do not add any more DDT to the environment it will be a decade before the full impact is felt on sea foodchains.

Ehrlich is no mean speaker. Words poured from him like steam from a boiling cauldron. But it was no evening for a scientist. Ehrlich was there to convince rather than to prove (what else could he do at a public meeting?), and when the going got tough he exhorted his audience "if you don't understand it, take it from me".

Turning to what can be done in the face of such a crisis he said that the UK may feel pretty insignificant with a population of 50 million among the 3,700 million of the world. "But England may hold a key, maybe the key", to the problem. Her contribution would be a psychological one. The world still watches England for a lead, he said, and any decisions taken, such as to cut the population back to 30 million, would have a big effect around the world.

Again, we must do something about the automobile before it finishes us off. All cars must be banned from central London and we must reduce demands for power—for example by changing the rate structure for electricity so that the more you use the more you pay per unit.

But all this is going to hurt. It cannot be done other than by producing a redistribution of wealth throughout the world. We must face the fact that "the pie is finite and some people have a disgustingly large share of it". Britain could lead the way and is more likely to than America, in Ehrlich's opinion. He was encouraged by Prince Philip's stand on the environment, by Philip Whitehead's vasectomy bill and the signing of the *Blueprint for Survival*—"that brilliant well-done thing in the *Ecologist*" but discouraged by that "pathetic" and "atrocious editorial in *Nature*" and by the BBC's refusal to rescreen the programme "Owing to the lack of interest, tomorrow has been cancelled".

Nonetheless, he told the meeting, "You're on your way and the bulk of the people are with you". But society must take corporate action and that meant involvement in politics. There must not, he warned later, be a "them and us" attitude to politicians and in-

dustrialists—"not every Rockefeller is an enemy".

The chairman of the meeting Mr Peter Scott of the World Wildlife Fund. In his opening remarks he said that "the most important problem in the world is how to safeguard our life systems". He had been encouraged, he said, by the publication of the *Blueprint for Survival*, and by the declaration published in *The Lancet* and the *British Medical Journal*, and "saddened by the unhappy counterblast from that distinguished journal *Nature*. I don't think, when you leave this hall tonight, that you will have any doubt who is right."

ENVIRONMENT

The Profit Carrot

PRESENT concern about the environment must be met by more, not less, production—but production of the right goods and services. This was the thesis of Dr David Hertz, Director of Technological Development of McKinsey and Company, New York, speaking at a Science Policy Foundation meeting in London last week. The meeting, which met to consider the ecological challenge to science-based industries, heard Dr Hertz express his belief that it is the science-based industries which can and must answer the challenge of ecological problems by redesigning their products to do less damage to the environment and by recycling waste energy and waste matter.

Dr Hertz argued that industry must change today's production line of materials, products and waste into a loop in which products and waste can be recycled. The Dow Chemical Company expects to make a profit from cleaning up pollution and Dr Hertz sees this as the carrot which will ensure industry's cooperation.

For Dr Hertz, at least, the road to survival lies along the use of much more technology to overcome the problems of waste and pollution. "The call to arms may be the job of amateurs," said Dr Hertz, "but the work to be done is not."

In conclusion, Sir Eric Ashby, chairman of the Royal Commission on Environmental Pollution, said that serious though the problems may be, scientists must not be irresponsible in their attitudes to it. He pleaded for "one more

SRC

MRS MARGARET THATCHER, Secretary of State for Education and Science, announced last week the appointment of Sir Alastair Pilkington to the Science Research Council. Sir Alastair will replace Mr D. L. Nicholson who has resigned following his appointment as chairman of the British Airways Board.

attempt" to overcome environmental problems by better use of present resources—for example, by building cars to last fifteen years rather than four. The factor in pollution which chiefly concerns the Royal Commission, he said, is the impact that each unit of goods and services produced has on the environment. It is the belief of the commission that reducing this impact, either by new production methods—as has happened in the case of steel and ammonia—or by legislation, could be a key factor in limiting pollution. To alter that parameter alone will not change the future, said Sir Eric, but at least it would be a constructive policy with which to face the problems.

TECHNOLOGY

Planned Futurology

ARTHUR D. LITTLE has acquired 80 per cent of the assets of Cambridge Consultants Ltd, the British contract research organization which started out as a one-man organization in 1960.

Cambridge Consultants is a company to gladden the heart of a Rothschild, for its sole source of income is contract research and development work. Last year its turnover was in excess of £200,000 and the company has shown a profit for the past three years—in spite of its connexion with AIM Associates Cambridge Group, which went into the hands of the receiver in 1971.

The link-up is expected to be mutually beneficial. Cambridge Consultants feels that as it grows from a small to a medium-sized company it could benefit from the organizational advice and contacts of an experienced management consultancy, particularly one that already specializes in high technology fields. Arthur D. Little sees CCL complementing its operations in Britain, forming a base within Europe, and co-operating with its American technological and research divisions. Indeed, although ADL has acquired 80 per cent of CCL's assets, at a cost of £100,000 including the injection of working capital, the link-up has more the air of a merger than a takeover. CCL will continue to operate under its present management, with its expertise made available to its American parent.

In the past, Cambridge Consultants has worked on projects as diverse as carpet looms and stereophonic amplifiers, teaching machines and radiation pyrometers, as well as defence work. One-third of its work is undertaken for the government and two-thirds for industry. With the experience and new capital of ADL behind it, CCL expects early expansion—up to 20 per cent next year, when turnover should exceed £250,000. Richard Cutting, CCL's managing director, believes that the proposals in the Rothschild report, if

Independent Contract Research Organizations in the U.K.

	Turnover (£thousands)
Huntingdon Research Centre	2,000
International Research and Development	1,100
Robertson Research International	740
Ricardo and Co.	740
Fulmer Research Institute	360
Inveresk Research International	250
Yarsley Research Laboratories	230
Cambridge Consultants	200

adopted, can only be to CCL's advantage, and predicts a "dramatic growth in contract research".

Certainly with the expertise of ADL behind it, Cambridge Consultants could well cease to be the comparative baby of the independent research organizations in Britain (see Table). The increase in contracts which CCL has received from blue chip organizations such as ICI, British Oxygen, BAC and Honeywell, the possibilities that the European Community will offer, and the combination of management consultancy and high technology could give the two companies the kind of success that ADL has enjoyed in America.

EUROPEAN SCIENCE

Doubling up Exchanges

THE number of postdoctoral and higher research awards for British scientists to carry on further study in Europe should be doubled, according to a Council for Scientific Policy working party under the chairmanship of Sir Harold Thompson. This increase should be brought about by expanding the present Royal Society European Fellowship scheme which in 1969 resulted in 198 scientists coming to Britain and 150 British scientists going to European

laboratories for further study.

The working party recommends increased collaboration within Europe because it feels that "a period spent working abroad at an early stage of [a scientist's] career should be regarded as a highly desirable feature of his training". According to the working party, collaboration with European laboratories is even more desirable now than it was in the past because of the cutback in research funding in North America.

Since 1945, most British scientists who have gone abroad have worked in North America and it is only in the past few years that there has been a significant number of scientific exchanges with European laboratories. But with the advent of Europeanism—exemplified by the setting up of the European Physical Society and the Royal Society European Programme—scientific traffic between Britain and the rest of Europe has rapidly increased.

Between 1966 and 1969, more than 1,100 awards were made by various organizations for scientific exchange within Europe, and half of these were provided by the Royal Society. These include both fellowships and study visits and the working party felt that the average of 85 outgoing fellowships awarded every year to British scientists is too small.

The financing of the Royal Society scheme has, however, received a setback during the current year (1970-71) because £40,000 from private funds has been spent during the past three years, and money from private sources is no longer available. The result is that the £255,000 now available is only slightly more than the £250,000 spent last year. Since the Royal Society scheme was started in 1967-68, the total annual expenditure has increased from £110,000 to the present amount. The Department of Education and Science matches the total contribution of the fourteen European countries involved in the scheme with a balancing contribution to the Royal Society.

Awards made by the Royal Society, the Research Councils and EMBO

	Fellowships		Study visits	
	From Britain	To Britain	From Britain	To Britain
Royal Society European Programme	145	122	208	76
Agricultural Research Council	—	—	26	—
Medical Research Council (own funds)	2	11	39	—
(paid by others)	14	—	24	—
Natural Environment Research Council	2	—	21	—
Science Research Council (with NATO)	86	4	6	56
NATO (European countries other than Britain)	—	257	—	38
European Molecular Biology Organization	5	1	20	—
Total	254	395	344	171

NEW WORLD

HEW Looks to its Own

by our Washington Correspondent

As the defender of equal employment opportunities and the scourge of discrimination in higher education, the Department of Health, Education and Welfare has a special responsibility to keep its own house in order. That is one reason why Elliot L. Richardson, Secretary of HEW, in February last year set up a commission to help combat discrimination against women employed by HEW and in the programmes sponsored by the department. Called the Women's Action Program (WAP), the commission last week published findings which show good reason why the department should be as vigorous in its pursuit of justice in its own ranks as it is in its attempts to stamp out discrimination in the universities and elsewhere.

Based on investigations conducted between March and September last year, the WAP report documents a host of alleged discriminatory practices in HEW which add up to a situation in which women employees are crowded into the bottom grades, while men carry off the cream of the responsible and well paid jobs. In detail, women make up about 63 per cent of the department's employees—the highest percentage of all federal departments—but their average government service grade is 5.8. Men, on the other hand, enjoy an average grade of 10.9—a gap of five whole grades in a scale that only runs from 1 to 18. And, to underline the difference, women hold only 14.4 per cent of the jobs in grades 13 to 18 while men occupy 85.6 per cent of the top leadership positions, and no woman has reached grade 18 in HEW.

Such figures are sufficient to make every feminist flush with anger and start to blame male chauvinism. But

unfortunately the cause and solution to the situation are much more difficult to diagnose than the effects. For one thing, as the report makes plain, the situation in HEW is simply a reflexion of the distribution of men and women among lower and higher paid jobs in society at large. A prime cause is that secretaries and similar support staff tend to account for many of the lower graded jobs in government service, and secretaries usually tend to be women; and a prime obstacle is that for a significant change to take place, attitudes towards the employment of women (among men and women) must change.

With the caveat, however, the report suggests a string of changes that should be made by HEW to promote upward mobility of women employees and a more liberal attitude towards such reforms as day care facilities and maternity leave. A significant step had already been taken with the creation last spring of the Office of Upward Mobility—an office charged with helping all HEW employees to move upwards through the hierarchy by means of retraining, counselling and revamping of the personnel structure. Since women account for some 80 per cent of the seven lowest grades in HEW, this office will probably be of greatest benefit to them. One of the chief goals of the office, the WAP report suggests, should be to help qualified secretaries and staff assistants to move into administrative positions. Too often, their advancement is tied closely to that of their immediate boss.

As for the programmes and the research and education supported by HEW, the report has a few recommendations to make. For example, the National Institute of Mental Health should sponsor more research into the status of women in American society, the biological and social determinants of sex differences, children's sex and

social role development, the psychological and social effects on a female rape victim and so on. The WAP report also points out that the Health Manpower Bureau of the National Institutes of Health has a potentially powerful role in overcoming sex discrimination in the health professions, and suggests that it should develop five and ten year goals to increase the number of women in medical and dental schools. Research into male contraceptives and other family planning methods are two obvious candidates for HEW sponsorship which the WAP report does not pass up.

Will the report do more than document the present situation before ending up in the waste baskets? There have, of course, already been suggestions that the Women's Action Program was set up chiefly as a convenient way of heading off criticism both from inside the department and from the universities which have been subject to action from HEW's Equal Employment Opportunities Office for their own discrimination. But Richardson has already asked the heads of HEW agencies to study the report and develop timetables to implement the recommendations pertinent to them. He also said at a press conference last week that he felt recommendations designed to increase opportunities for part time employment and day care facilities are most important.

SOVIET UNION

Lerner's Battle

by our Washington Correspondent

THE short detention and subsequent expulsion from the Soviet Union last week of Mr James H. Scheuer, a Congressman from New York, has brought to public attention the name of Professor Alexander Ya. Lerner, Scheuer's host the night he was detained by Soviet police. Lerner was one of the six or seven Soviet Jews visited privately by Scheuer during his visit to the USSR as part of a Congressional study group looking at educational institutions, and it was those visits that earned his expulsion on the grounds of improper conduct. Lerner is, however, perhaps the most important of the Soviet Jews visited by Scheuer, and his plight has received attention from scientists both in the USSR and in Western countries.

A distinguished scientist with an international reputation in cybernetics, control theory and systems theory,

Table 1 Median grade and distribution of men and women in agencies of HEW July 1971

Agency	MEN			WOMEN			difference in median grade
	Number in Agency	per cent of agency	median grade	number in agency	per cent of agency	median grade	
SSA	18,260	32.9	9.6	37,245	67.1	5.1	4.5
HSMHA	6,627	34.5	10.3	12,580	65.5	5.8	4.5
NIH	3,778	41.4	11.5	5,345	58.6	6.9	4.6
OS	1,500	45.0	13.9	1,831	55.0	6.9	7.0
FDA	2,911	62.1	12.3	1,775	37.9	6.1	6.2
OE	1,500	45.0	13.9	1,831	55.0	6.9	7.0
SRS	834	41.3	13.9	1,185	58.7	6.9	7.0
Total	36,443	36.9	10.9	62,288	63.1	5.8	5.1

SSA=Social Security Administration; HSMHA=Health Services and Mental Health Administration; NIH=National Institutes of Health; OS=Office of the Secretary; FDA=Food and Drug Administration; OE=Office of Education; SRS=Social and Rehabilitation Service.

Lerner was dismissed from all his professional positions in October last year soon after he applied for a visa to emigrate to Israel. Until then he had been Director of the Department of Large Scale Systems of the Institute of Control Sciences, and a Professor at the Scientific and Technical University in Moscow.

According to scientific colleagues in the West, Lerner's dismissal from the institute and the university came after two months of abortive attempts to obtain a visa to emigrate. Early in September, his relatives in Israel sent him an invitation to emigrate there, but the letter was intercepted and never reached Lerner. On September 16, Lerner was asked by Academician Trapeznikov, Director of the Institute of Control Sciences, to explain the letter, and Trapeznikov called in directors of all the departments and laboratories of the institute and informed them of the invitation.

Towards the end of October, however, Lerner received a duplicate copy of the invitation, and went ahead with a formal application for a visa. (A letter of invitation is required before application can be made.) Soon after that, he was dismissed from the institute and the university and prevented from teaching. His son, who was also working at the Institute of Control Sciences, was also fired.

So far, a number of letters of protest written to Academician Keldysh, President of the Academy of Sciences of the USSR, by Soviet and Western scientists have failed to get Lerner permission to emigrate.

UNEMPLOYMENT

Prescription Needed

by our Washington Correspondent

At this time of year, industrial talent scouts usually start to comb the campuses for promising potential graduates to sign up. But this year, like last, is seeing a marked drop in the number of recruitment officers on campuses, and most of those who are seeking new talent say that they have fewer jobs to offer than usual. The prospects are once again that the supply of educated manpower coming out of the universities this year will exceed the demand, and in spite of predictions from the Department of Labor that in the 1970s as a whole college output will match the number of jobs available, an increasing number of critics are urging that the government should adopt a policy to prevent the present manpower crisis from developing into a chronic unemployment situation.

One forceful advocate for policies for educated manpower is Eli Ginzberg, professor of economics and director of the Conservation of Human

Resources Project at Columbia University. Writing in the winter 1972 issue of *Public Interest**, Ginzberg warns that "there is no reason to believe that the economic recovery will have a major effect in absorbing all those now looking for positions as well as those who will be coming into the market". He bases this suggestion on the fact that there is likely to be little change of direction in the federal budget, and that the precarious financial position of many colleges and universities will cause them to cut down on the hiring of new staff. Private industry is therefore the chief remaining source of demand for education manpower, and this, Ginzberg suggests, will not grow sufficiently to take up the slack from the public sector.

One constant pressure to alleviate the manpower situation is to reduce the supply—the federal government has already cut back on student support, and New York State has recently placed a one-year moratorium on

approval of new doctoral programmes. But Ginzberg warns that "we can no longer rely on the decision making of individuals, the blind responses of the educational system to pressures for admission and the cues of the market to provide the country with the educated manpower it needs and to assure that it will be effectively deployed".

What is needed, he suggests, is a long-term policy for federal support of science with regard to both level and rate of growth—since the federal share of the research and development budget is about 60 per cent, erratic fluctuations lead to serious manpower distortions—a policy for support of higher education that will keep solvent the 100 or so chief university centres and provide support for graduate students, and to take account in federal budget-making of the manpower implications of big projects.

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Short Notes

Interracial Living

A REPORT published by the National Research Council—the operating arm of the National Academy of Sciences—suggests that Americans are becoming more racially tolerant. The report suggests that the proportion of white Americans who say they would not mind having black neighbours has more than doubled between 1942 and 1968 (from 35 per cent to 76 per cent), and that "for both blacks and whites the quality and convenience of housing and neighbourhood services take precedence over racial prejudice in housing decisions". But in the same breath, the report points out that a smaller proportion of minority groups now live in racially mixed neighbourhoods than was the case thirty years ago. The reason for the paradox, the report suggests, is that white Americans attribute class characteristics to race, and integration is hindered because of class prejudice rather than racial prejudice. "It is this racial perception and not race *per se* that leads to resistance", the report notes. In other words, integrated housing is still hindered by prejudice. (Report of the Advisory Committee to the Department of Housing and Urban Development, National Academy of Science.)

Scientists for McGovern

A small group of scientists, headed by four Nobel Laureates, has formed an organization to assist Senator George McGovern's bid for the Presidency of the United States. In a statement issued last week, the group charged that the present Administra-

tion's handling of science and technology is "obtuse, incompetently managed, distorted by political motives and wasteful of expert resources". The group cites in particular the shuttle as a pointless waste of resources, and urges more international cooperation on such research as fusion technology and solar power. Scientists for George McGovern, as the group is called, plans to support the campaign by providing technical advice, and by soliciting support from fellow scientists. The Nobel Laureates associated with the group are O. Chamberlain, S. E. Luria, A. Szent-Gyorgyi and George Wald.

Mansfield Opposes Shuttle

Senator Mike Mansfield, Senate majority leader, has thrown his considerable political weight behind the movement to oppose funding for the space shuttle. Calling the project "a misplacing of priorities", Mansfield said that the "public is a good deal smarter than it is given credit for being".

Marine Pollution

The Ocean Affairs Board of the National Research Council has put forward a plea for urgent studies to be made of toxic materials, particularly polychlorinated biphenyls, heavy metals and radionuclides, and their impact on the marine environment. Emphasizing the fact that concern about the environment is not matched by adequate research and information gathering, the board recommends five chief areas of study: identification and routes of harmful materials; dispersal; biological and geochemical transfer, effects on man and other life organisms, and sites of final deposition.

No Need to Change a Winning System

A shortened version of the views of the Directors of Research Institutes and Units of the Agricultural Research Service on the Rothschild proposals.

- Accepting the customer-contractor principle for applied research development, several principal questions still require consideration. To maintain and improve the basic materials on which agriculture and food depend—the soil and breeding stocks of animals and plants—is a long-term commitment of agricultural research and, moreover, one of the most important aspects of environmental conservation for posterity. Because of this, national requirements cannot be met without a substantial proportion of long-term research and the customer organization must represent at least three largely independent interests—the agricultural industry, the consumers of agricultural produce, and in a broader sense the entire community now and in the future—interests which involve other organizations and other government departments than the Ministry of Agriculture, Fisheries and Food (MAFF).
- Problems and priorities have hitherto been identified partly through farmer and MAFF representation on the council and on governing bodies and advisory committees, partly through many technical committees on which the industry and the MAFF are represented, and partly through informal contacts and our own professional assessment of the industry's needs. The machinery needs strengthening—action with that aim being already in progress—but we do not believe it would be helpful to interpose a single new agency between ARC and the real customers for its research.
- Responsibility for allocating tasks between and within research centres must remain with the ARC, free from interference by ministry officials. Experience proves that administrative ability is not sufficient to direct research.
- It is moreover important that ARC research be protected from administrative pressures and we cannot agree that such considerations “have little or no bearing”. There could be no protection if an executive ministry controlled a substantial part of ARC funds and some form of intervention by an independent authority would be essential.
- We agree that the present scientific strength of the MAFF is inadequate for the functions envisaged, and that a new “Chief Scientist Organization” would need to be created, before introducing a system in which that ministry was the principal customer. We welcome the proposal to appoint a Chief

Scientist. But it will take him several years to assess the situation and establish himself so as to have the confidence of farmers and ARC staff. Hence it cannot be advantageous for him precipitately to assume control of research contracts.

- In implementing the customer-contractor principle, it is crucial to appreciate the following. First, three different types of research were distinguished by the CSP, tactical, strategic and basic, the first representing short-term applied research. The research and development contracts recommended by Lord Rothschild should apply only to tactical research (compare the suggestion in his appendix (ii) that the programmes of institutes which “come into the category of what is sometimes called ‘strategic research’ should be excluded from the ‘customer-contractor’ relationship”). The ARC estimates that about 20 per cent of the ARC funds now go to tactical research and development, 60 per cent to strategic research “concerned with the understanding of an agricultural process”, and 20 per cent to basic research. Second, Lord Rothschild's proposals would have the effect of raising the proportion for applied research and development to 78 per cent (£14.5 million out of £18.5 million), including a 10 per cent “surcharge for general research”. The remaining 22 per cent is to be spent on units in universities and on strategic research (that is, “research in a field of agricultural interest, but without an applied objective which is likely to be realized in a specified time, however long”) in five institutes, at only two of which basic research is mentioned (appendices (ii) and (iii)). Besides restricting long-term research virtually to units and a few institutes, such proposals mean that almost the entire programme of most institutes must consist of short-term research and development projects with little of their present long-term strategic content.

- In contrast to Lord Rothschild we think that strategic research has long-term practical objectives, being essential for the understanding of applied problems, and hence much of it should be undertaken by the ARC. Sufficient continuity of purpose can seldom be assured in university departments. Moreover it is our experience that a well balanced mixture of basic, long-term and short-term applied research is highly de-

sirable (compare Rothschild, paragraph 39) to attract the most able scientists and stimulate maximum creativity, and should be maintained in any circumstances. We know the mutual benefit to each when the three types of research are carried out in the same place by the same workers. Therefore, we emphatically disagree with restricting basic research to certain institutes while confining other institutes to applied work. Similarly, we strongly disagree with Lord Rothschild's suggested overall balance between the types of research. In the national interest, there is a need for a substantial volume of strategic agricultural research which, if not done by the ARC, will not be done at all. Lord Rothschild has made no case for diverting the ARC budget for this purpose to contracts for short-term problems.

- Developmental work, as distinct from research, has received little mention in spite of its vital importance. Lord Rothschild proposes that the customer should have greater than usual financial control, but appears to suggest (paragraph 28) that control should be by the chief executive of the research council. In the agricultural field, however, one would suppose development to be the function of the Agricultural Development and Advisory Service of the MAFF. Development work has nevertheless been a significant part of the ARC programme, though it should be better integrated with similar ministry activities in which not enough help has been invited from ARC scientists. We consider that this integration would be promoted by transferring control of the ministry's experiment stations to the research council.

- The Rothschild report takes no account of the fact that the high standard of agricultural research depends critically on the quality and enthusiasm of our staff, both of which are excellent. We view with concern proposals whereby ARC income might be reduced by 27 per cent over 4 years with possible further reductions thereafter, because this must cause the dismissal or loss of many staff, which would destroy the confidence of the remainder and discourage them from studying any but the most immediate problems.

- To conclude, we refer to the conclusion of Lord Rothschild's report: “The recommendations made in this report cannot be implemented overnight”. They cannot. The practical way to improve the structure of agricultural research, without detriment to progress, would be to strengthen the existing ARC structure in close consultation with the Agricultural Departments.

The Framework and the Fabric of Agricultural Research

Professor Ralph Riley, Director of the Plant Breeding Institute at Cambridge, argues that a substantial amount of agricultural research must necessarily be carried out on a long term basis, in contradiction to Lord Rothschild's proposals.

THE uninitiated might infer from *A Framework for Government Research and Development* that the research councils have failed to deliver the goods packaged in forms that produce economic benefit to the community. This is demonstrably not true for a great deal of the work of the Agricultural Research Council, and it would be a serious injustice to many past and present members of ARC staffs if their achievements were disregarded so disdainfully. In the field of plant breeding, with which I am most familiar and to which reference has been made frequently in recent weeks in the columns of *Nature*, the economic benefit has been considerable.

For example, in the wheat crop, which was worth about £120 million in 1971, two recently introduced varieties yield 9 and 10 per cent more than any other available variety. If only part of this is realized in farm practice, this ARC-supported programme will obviously have been effective, particularly since it also produced two other widely grown varieties.

In 1968, Dr G. D. H. Bell was the first recipient of the Mullard Award of the Royal Society, which is given for work that has led directly to an increase in the national prosperity of Britain. The impact of Bell's barley variety Proctor, in adding 15 per cent to the yield of the crop, whose current value is about £150 million a year, was judged to be greater than that of any other scientific innovation considered, which presumably spanned all aspects of engineering.

Not all of the ARC's plant breeding has been equally successful, but the overall attainment has been good and certainly the work has been relevant and responsive to economic needs. So we can ask what will be the effect of changing the pattern of support and control. Because there are no adequate reserves of research capacity elsewhere in Britain, gross interference or experiment with state-supported plant breeding would hazard an economically important asset. Nevertheless those of us working in this sector are not opposed to change and we do believe that we can improve our ways of working and organization. Indeed a searching public discussion that would diagnose

present defects would be beneficial. This could lead to logical remedies in the form of a number of models of improved organizational arrangements. These would be compared and successively eliminated, like the models set up to explain any complex biological system, until the best fit was obtained. When only one model—like that of Lord Rothschild—is offered, in spite of the well-meaning efforts of the Dainton Committee, it inevitably becomes an Aunt Sally because there is no other target. So the illusion is created that the scientific community is opposed to change when it is reacting principally to the limitation on its freedom of choice.

Hobson's choice is offered by Lord Rothschild, so let us see what would be the effect of the application of his model on a successful and economically relevant research sector. The customers of ARC-supported plant breeding are the farmers who choose to grow our varieties, the crop processors and the community at large. Clearly, individual farmers are not able to sponsor research, which is why agricultural research has a different structure from that of any other industry. These customers, however, have always played a part in formulating our programmes by service on councils, committees and governing bodies and also in informal ways. Only the farmers can say whether the MAFF as a monopoly customer of ARC research could represent their interests adequately.

Now we can turn to the nitty-gritty of the Rothschild proposals and their application to a particular research field. Crop variety production involves long-term programmes that often last at least 10 years and that cannot be subdivided into short-term components. Moreover, success or failure is not apparent until the final stages, so plant breeding programmes would only be amenable to the open-ended type of contract against which customers are warned by Lord Rothschild. In addition, it is not possible to separate applied plant breeding from basic research, even following the Rothschild definitions. Thus, unless we discover the "rational correlations and principles" (para. 7), involved in the interactions of the genotypes of plant hosts and pathogenic organisms, we cannot

rationally breed disease-resistant varieties. The success of ARC plant breeding has derived from the intimate association of basic and applied research, and the part of the total programme that must be devoted to basic research is very much greater than would be provided by the 10 per cent surcharge. Without the back-up provided by this basic research, the techniques and methodology of applied research would not advance. All would then suffer because it is ultimately methodology and research competence, allied to scientific experience and judgment, that a customer of applied research would purchase.

The principal difficulty in attempting to operate a customer-contractor system, based very largely on short-term contracts, would be the inadequate opportunity offered for the forward planning of research facilities, especially when the contractor had no certainty of continued support. No commercial contractor could operate on this basis; his market research would have shown him the nature and scope of the market and he would equip to deal with it. Building a research and development organization in agriculture requires long-term planning of resources of land and buildings and of large scale capital facilities. Most important, long-term planning is necessary to provide a properly structured staff which can only come from appropriate recruitment and training policies.

One approach would be to ascertain the budget needed to sustain a stable research service of the necessary capacity, competence and flexibility. This budget could be agreed on a long-term basis, say of 10 years, and to it could be added funds from contracted research which should form not more than 20 per cent of the total income.

The stability of the research programme and the assured continuity of the research service so provided would allow us to retain and attract staff of high calibre who would be lost to an uncertain system committed entirely to short-term contracts. This is important because in plant breeding, as in all other research, the source of innovation is the individual worker and the research team. From this it follows that whereas it may be reasonable for the MAFF to have a voice in formulating research policy, unless a major share in determining programmes is left in the hands of the research workers involved, enterprise and originality will be driven from government research and development. The framework that might then exist would not be clothed in the fabric of creativity and imagination without which it would be a useless structure.

NEWS AND VIEWS

Steroid Receptors and Gene Expression

STEROIDS are small and rather simple molecules which nevertheless elicit a complex array of biochemical responses in their target tissues, often leading to profound changes in growth and differentiation. Exactly how they achieve this is still not known but there is very good evidence that the first step of crucial importance is the specific association of steroids with molecules of higher informational content, namely cytoplasmic protein receptors. Steroids thus differ radically from polypeptide and other hormones which combine with membrane-associated receptors and stimulate adenyl cyclase to produce cAMP. Several recent publications, including the article by O'Malley *et al.* on page 141 of this issue of *Nature*, have shown that the study of steroid receptor proteins is just beginning to reach a point where interesting and speculative ideas about their biological role can be tested.

Although there are usually several classes of receptor proteins for a particular steroid (defined as proteins with high affinity and low capacity for the steroid) in the cytoplasm of its target tissue, there is always one major species with very characteristic properties. When the cytoplasm is prepared in the presence of 0.3 M K^+ this species sediments in a sucrose gradient in the same buffer at around 4S. When K^+ is omitted this binding protein now sediments at around 7–8S, either as a result of a conformational change or because of association between subunits or association with another cytoplasmic protein. (The exact state of the receptor *in vivo* is not known because a gradual reduction in sedimentation behaviour is seen between 0.15 and 0.3 M K^+ .) This reversible, salt-dependent, 8 to 4S transition is a common property of the principal oestrogen receptors in the uterus, the cortisol receptors in liver and lymphoid cells, and the progesterone, dihydrotestosterone and aldosterone receptors in their respective target organs.

There is another, much more interesting, property common to this class of receptors. When combined with steroid they are taken up in a step, which is temperature and possibly energy-dependent, from the cytoplasm into the nucleus, where they are accumulated. The steroid-receptor complex can only be extracted from the nucleus or chromatin with high salt (0.4 M K^+) and in these conditions it sediments at 4–5S (the actual sedimentation coefficients vary between receptors and are, in any case, only approximations). The fact that the hormone-receptor complex can only be extracted with high salt suggests that it is quite tightly bound to the chromatin. The uptake and binding of the cytoplasmic hormone-receptor complex can be observed in cell-free systems, and many such experiments *in vitro* have shown that the complexes are specifically accumulated in nuclei and chromatin from target tissues and not in the corresponding fractions from non-target tissues.

The steroid receptor:nuclear interaction promises therefore to be a very elegant system in which to study the role of specific cytoplasmic proteins in the control of gene expression, and many important questions beg to be

answered. For example, is the steroid-receptor complex modified during nuclear transport; does it bind to DNA itself or to other DNA-associated proteins or to both; how many steroid-receptor binding sites are there; how tight is the association? And so forth. Although almost nothing is known about the others, there is some contradictory information about the second question.

Work with the oestrogen-receptor complex isolated with high salt from uterine nuclei (King *et al.*, *Advances in the Biosciences*, 7, 21, Pergamon Press, 1971) has suggested that this complex will reassociate *in vitro* with chromatin only from oestrogen target organs, but will bind to purified DNA from both target and non-target tissues. This finding is compatible with steroid-receptor binding to DNA but these sites are masked by other proteins in non-target chromatin. O'Malley, on the other hand, working with the cytoplasmic progesterone-receptor of chick oviduct, claims that there is more extensive binding of receptor to target tissue chromatin than to purified DNA; this binding is enhanced by the presence of a particular class of acidic proteins. This finding is obviously more compatible with steroid-receptor binding to a second protein, or to DNA sites which are only held in the correct configuration by non-histone proteins. Clearly, more systems must be analysed before any firm conclusions can be reached about the nature of the steroid-receptor binding site and in their absence speculations about the function of the receptor complex, once it is bound, are almost free of constraint. Thus, by binding to DNA, it could either be acting as a classical *lac* repressor, inhibiting the synthesis of RNAs coding for other repressors, or it could be opening up regions of double-stranded DNA and enhancing the transcription of RNAs perhaps coding for a few key proteins which then switch on other genes. Binding to protein rather than to DNA is more in line with a role as a sigma-like factor or anti-termination factor of RNA polymerase.

Whatever the reason, the fact that the hormone-receptor complex is unable to bind to non-target chromatin means that responsiveness of a target tissue to its steroid depends on more than the presence in the cytoplasm of the steroid receptor proteins. Indeed, there is evidence that the acceptor property of target tissue nuclei may be modified by different physiological conditions of the animal.

It will be clear from the foregoing discussion that testing the various hypotheses for the nuclear role of the steroid-receptor complexes will be no easy task and will almost certainly require the isolation on a larger scale of the complexes and greater purification than has so far been achieved. This will also provide much important information about the number of steroid binding sites and the subunit structure *in vivo* of the receptor molecules. For example, in his article in this week's *Nature*, O'Malley provides evidence that the cytoplasmic progesterone-receptor which has been quite extensively purified from chick oviduct may consist of two subunits, both capable of binding steroid but only one able to bind to chromatin.

Hopefully, it may also be possible to obtain more direct evidence for a conformational change in the receptor protein on binding steroid. Indirect evidence is available (Samuels and Tomkins, *J. Mol. Biol.*, **52**, 57; 1970) that the cortisol receptor may exist in two different configurations, one active in eliciting enzyme induction, the other inactive, and that different steroids may stabilize different states. Thus certain cortisol analogues, by competing for the binding of cortisol but stabilizing the inactive state, behave as "anti-inducers" similar to ONPF (ortho-nitro-phenyl-fucoside) which competes for IPTG binding to the *lac* repressor but increases the affinity between the repressor and operator.—B. H.

Afar and Iran

Two articles—by Tazieff *et al.* and by Takin—on pages 144 and 147 in today's issue of *Nature* provide further elements in the jigsaw puzzle of plate tectonics in the Middle East. Although plate tectonics was developed as a theory to explain, very successfully, the motions of less than ten large plates each of many thousand kilometres in extent, there was a hope that in more complicated regions such as the Middle East and the Western Pacific the same principles would apply. Specifically, one could ask the question whether plate tectonics works as well for small plates less than a few hundred kilometres in extent and for movements as small as a few tenths of a millimetre a year. Can seismicity still be used to delineate plates and can the plates still be regarded as rigid? The answer is not obviously yes. Seismicity maps in some regions of the world show scattered activity, particularly on continents. There is also evidence that seismic observations over the past 50 years represent an inadequate population on which to base tectonic conclusions in some regions where motion is slow. So although the large plates have been very successfully delineated with the aid of seismicity, some of the smaller plates still need careful study.

When seismicity is inadequate for answering detailed questions, the chief alternative is to attempt to infer from geological observations what the present and past tectonic situation is, and the articles by Tazieff *et al.* and by Takin are both concerned with geological evidence. Geology being so detailed is not the most reliable of indicators of plate tectonic character, and so it has to be used with some discretion. A great strength of marine geophysics in developing modern theories of tectonics has been the necessarily smeared-out view it gave of the crustal structure of oceans. This allowed a relatively uncluttered approach to large-scale problems, and the geologists who have most readily capitalized on the new ideas have been those who knew where to draw the line at attention to detail.

Tazieff *et al.* are concerned with one of the more persistent problems in application of plate tectonics in "low-velocity" situations, the nature of the Afar depression. Afar is a triangular region at the junction of the Red Sea, Gulf of Aden and East African Rift. Much of it is below sea level, although not submerged. The volcanic nature of the Afar fits in well with ideas of the region being simply a piece of exposed oceanic crust. There is, however, more to it. A sizable area to the east of the

depression is a horst—the Danakil horst—which could by no stretch of the imagination be termed ocean floor. This rather narrow finger stretches across from the Nubian to the Arabian plate at an unusual angle, almost giving the impression that the two ends are loath to let go of the separating plates.

Tazieff *et al.* go for a relatively simple explanation of the region as a corner in the ridge system which runs directly from the Red Sea to the Gulf of Aden with the Danakil horst as a continental fragment. They do not see the East African Rift as having any close relationship to the Afar region because the tensional features of the East African Rift do not continue into Afar. At this stage they come into headlong collision with Mohr (*Nature*, **218**, 938; 1968) and McKenzie *et al.* (*Nature*, **226**, 243; 1970) who for differing reasons have concluded that the East African Rift System is directly related to these other features.

McKenzie *et al.*, using standard plate tectonic techniques, concluded that there was a triple junction at Afar and that it was possible to predict the (very slow) spreading rate in East Africa. Mohr saw extensions of the East African Rift across Afar up to the Red Sea in the Wonji fault belt. Tazieff *et al.* see no evidence to support either of these points of view. The contentious issues are by no means finally resolved and the areas of disagreement are likely to be a battleground for some time to come. Lest, however, the armchair critic in his cosy laboratory views this disagreement as a sign of a weakness in the earth sciences, let it be said that the Afar is about as unpleasant a place to work in as is possible, and those who venture into it can risk their lives.

Just as there is little doubt that the Afar region has been the site of the generation of new oceanic crust, it is widely believed that there has been a continuous destruction of seafloor in the region of Persia. Both Africa (including Arabia) and the Indian subcontinent seem to have been separated from the main Eurasian landmass, at least until 70 million years ago. Since then these two blocks have moved independently northwards. India's collision with Eurasia produced the Himalayas as the motion was brought to a standstill and at a similar time a very complex collision occurred as the leading edge of the Arabian-African supercontinent also started to come up against Eurasia. This collision may be continuing today although the last remains of the Tethys Sea, the former ocean that separated Arabia from Eurasia, have now disappeared from view.

It is, of course, totally unlikely that two continental margins will, when brought together by plate motions, fit neatly together, so that it is necessary to envisage a period during which the two edges find a way of welding together most snugly. McKenzie has described such a process by which the Mediterranean is closing and ocean crust is being destroyed in order to bring continental plate tightly together. Takin, in the article on Iranian geology, envisages a comparable process of accommodation.

Takin also shows how the broader geological features of Iran fit well into the plate framework. The Zagros folded belt runs parallel to the Persian Gulf and shows evidence of steady quiet sedimentation from Cambrian to Pliocene times. He takes this region to be the continental shelf of the leading edge of Africa-Arabia. Recent folding has been evidence of the collision with Eurasia, and the ophiolites along the north-east of this belt are clear

markers of the region of ultimate plate consumption where oceanic crust reaches the surface. North of this ophiolite zone, Takin identifies distinct portions of Iran which he proposes were once separated microcontinents, but have been closed together by the general compaction of a mosaic in this region.

Takin's view of the timetable is that the microcontinents had been formed before the collision started by the opening up of small Red Sea-like embryo oceans, so although he puts the first engagement of the two major blocks in the late Cretaceous, for a long time thereafter the compression was taken up by a sort of extension process in which the small oceans were driven out. Only after this had been achieved did the folding in the Zagros region begin.—D. D.

Lasers and the Lamb Shift

EXPERIMENTAL investigations of the spectrum of the hydrogen atom have naturally been closely linked with significant advances in atomic theory because of the simplicity of the hydrogen atom. Any new spectral study of the atom which exceeds its predecessors in resolution is therefore likely to excite interest, and the elegant high resolution study of the $H\alpha$ line of the hydrogen atom by T. W. Hänsch, I. S. Shahin and A. L. Schawlow of Stanford University, California, in next Monday's *Nature Physical Science* (January 24) is thus attractive. Using the technique of laser saturation spectroscopy, they have observed the Lamb shift in components of the $H\alpha$ line fine structure for the first time (see figure).

The distinguished role of the hydrogen atom began when Balmer fitted its convergent series of lines in the visible region very precisely with an empirical formula. Later, Bohr was able to derive the Balmer formula theoretically, but only by using the startling postulate of discrete circular orbits for the electronic motion, which meant discrete energy states for the atom. Bohr's treatment suffered, however, from fundamental difficulties which were unresolved until Schrödinger's description of the hydrogen atom based on the wave nature of matter. Schrödinger, in fact, also predicted discrete energy states for the atom and the same energy level formula depending only on the integral quantum number n and some fundamental constants. The difference was that Schrödinger predicted several different states of electronic motion, not just one, to be associated with each n , so that each energy level was really a superposition of several. The Balmer $H\alpha$ line, arising as it does when photon emission accompanies the change of an atom between $n=3$ and $n=2$ states, is therefore several lines exactly coincident. A further extension of the theory by Dirac included the effect of relativity on the electron mass and predicted the energy level degeneracy to be lifted, resulting in a closely-spaced fine structure for the $H\alpha$ line. For some years, however, attempts to test the theory by resolution of the fine structure were frustrated by the Doppler width of the lines.

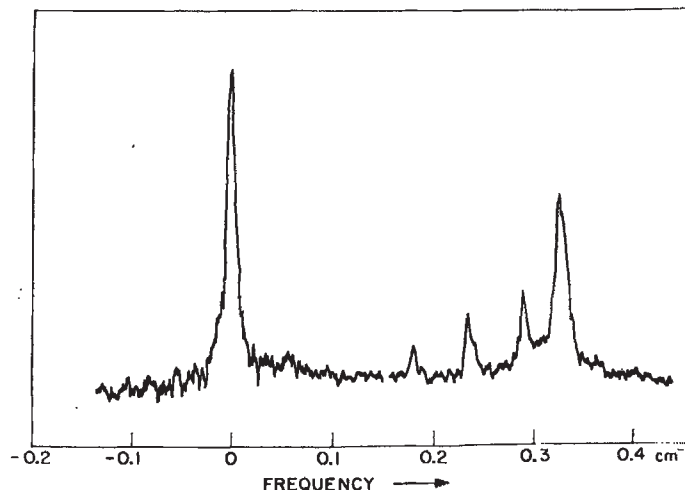
The Doppler width of absorption lines arises because atoms present a range of velocities relative to the direction of incident radiation. The wavelength at which absorption occurs is therefore shifted by the Doppler effect to

different extents in different atoms. Clearly, the shorter the incident wavelength the more serious the broadening. The hydrogen atom, being very light, has a wide velocity distribution and at the short wavelength of the $H\alpha$ line has an absorption profile broad enough to obscure most of the predicted fine structure.

The first conclusive test of the Dirac formula was a radiofrequency experiment by Lamb and Retherford. They observed directly the transitions between the fine structure components of the lower ($n=2$) level involved in the $H\alpha$ transition using microwave radiation, at which wavelengths Doppler broadening is unimportant. The levels were spaced almost but not quite as predicted, for one was shifted significantly relative to the others. In the successful theoretical interpretation which soon followed, this shift was attributed to the different interactions of the electron in the various states with the tiny residual electromagnetic fields always present in space. Further refinement in experiment and theory followed but the fine structure of the $H\alpha$ line itself remained unresolved until recently, when the problem was solved by the Stanford group using a technique rather simple in principle.

The offending Doppler broadening of the $H\alpha$ line is eliminated by use of laser saturation spectroscopy. Highly monochromatic optical radiation which can be tuned continuously over the range of the $H\alpha$ line is provided by a dye laser. The laser beam is split in two by standard optical techniques and the two components allowed to travel the same path but in opposite directions through atomic hydrogen gas. Only those atoms with nearly zero velocity in the radiation direction can simultaneously interact with both oppositely travelling components, otherwise one or other would be Doppler-shifted outside the very narrow natural line width of the absorbing transition. One of the beams is made powerful enough to saturate the transition in question so that absorption of the other, weaker beam by those atoms of zero relative velocity able to interact with both is decreased. The spectral feature observed on monitoring the probe beam has then essentially the natural width of the transition as this two beam trick has selected a very narrow band of atomic velocities and thereby eliminated Doppler broadening. The fine structure components of the $H\alpha$ line so resolved agree both in frequency and relative intensity with theoretical predictions.

Some experimental difficulties have been neatly solved. For example, to provide sufficient atoms in the lower,



Saturation spectrum of the $H\alpha$ obtained by Hänsch *et al.*

$n=2$, states of the transition, low pressure hydrogen gas must be subject to a gentle electrical discharge as it is pumped through the absorption cell. But electric and magnetic fields present during the discharge would broaden the transitions of interest. Optical observation is therefore delayed by electronic means until 1 microsecond after the discharge is switched off. Furthermore, two devices discriminate in favour of the atomic absorption and against noise signals. First, the probe beam is compared with a similar beam from the same laser which also traverses the gas but along a different, parallel path. The effects of random laser amplitude fluctuations are thereby reduced. Second, the saturating beam is amplitude modulated by a simple chopping method while the probe beam is amplified at the chopping frequency by a narrow band amplifier. This ensures that only absorptions due to atoms simultaneously subject to both beams are detected, because only then will the probe be modulated at the chopping frequency.

Advantages other than the further confirmation of important theoretical predictions should accrue from refined versions of this experiment. For example, as it is pointed out, a value of the Rydberg constant of accuracy comparable with the present standard of length should be obtainable. Because the Rydberg constant depends only on a few fundamental constants (one of which is Planck's constant), such an improved value would help one to a better knowledge of these important quantities.—A. C. L.

Chemotactic Signals

EMBRYONIC development is characterized by the organized growth, differentiation and movement of fields of cells. It is suspected that such global phenomena are controlled by embryonic systems of communication which may be analogous to the functional control systems in adult organisms. The nature of such communication remains an obscure and fascinating problem.

In last week's *Nature New Biology* (235, 90; 1972) and in a previous publication (*Biochem. Biophys. Res. Commun.*, 42, 119; 1971), Gerisch *et al.* report an investigation of a morphogenetic movement which is currently probably the best understood example of a field phenomenon—the aggregative movement of the cellular slime mould *Dictyostelium discoideum* in which free-living slime mould cells are induced on starvation to begin their development by aggregating chemotactically. Each aggregate ultimately forms a fruiting body (adult stage). The aggregation occurs by a movement response to periodic signals, which are propagated centrifugally through each aggregation field. It is almost certain that the signals are pulses of 3'-5'-cyclic AMP (cyclic AMP) and that they are propagated by a relaying system, whereby a cell which receives a signal responds by making one. The cells secrete an extracellular cyclic AMP phosphodiesterase (PD), which is traditionally considered to reduce noise in the system.

Gerisch *et al.* are concerned with regulation of the PD activity, and report that whereas suspension cultures of vegetative *D. discoideum* cells have a high extracellular level of PD activity, the activity drops in starved cell sus-

pensions. They have shown that this is caused by the appearance of an extracellular PD inhibitor which is a heat stable protein (molecular weight 40,000) and is specific in the sense of not inhibiting beef heart PD or another extracellular *D. discoideum* enzyme (N-acetyl glucosaminidase). The inhibitor appears one to two hours after starvation (aggregation occurs after eight hours) and causes a rapid fifty-fold drop in activity of the enzyme, which is followed by a slow rise (to ten-fold after eight hours).

Gerisch *et al.* examined twenty-three morphogenetic mutants of *D. discoideum* which failed either to aggregate or had unusual aggregation and six species of slime moulds related to *D. discoideum* for their PD-inhibitor systems. Twelve of the twenty-three mutants had defects in PD control (ranging from absence of PD or inhibitor to timing defects). In the three cases where the relationship between morphogenetic defects and PD-inhibitor defects is given, one mutant which failed to aggregate had no PD inhibitor and two mutants with giant aggregations had very low PD activity. Among the six species, three, including *D. discoideum*, which co-aggregate and presumably have similar movement control systems, had similar and cross-reacting PD-inhibitor systems whereas three which fail to co-aggregate with *D. discoideum* had either very little extracellular PD and no PD inhibitor (two cases) or an extracellular PD activity but no heat-stable inhibitor (one case).

These findings suggest that the PD has a vital role in the aggregation of *D. discoideum*. Gerisch *et al.* suggest that its primary role may not be to dissipate signal noise, but rather to control differentiation of aggregation competence by way of regulation of the external level of cyclic AMP. This function would be consistent with the time course of PD activity and with the observation that extracellular cyclic AMP (10^{-3} M) causes changes in *D. discoideum* cells which are associated with the aggregative and later stages of development (for example, it promotes cellular adhesion). Gerisch *et al.* report two more findings to support their contention: namely, that a cold shock at the end of the growth phase suppresses both inhibitor production and the onset of aggregation competence of cell suspensions of *D. discoideum* and that beef heart PD, which is not affected by the *D. discoideum* inhibitor, retards the onset of aggregation competence in the slime mould.

One criticism of this study is that the experiments have been performed on cells in liquid culture, whereas *D. discoideum* normally aggregates only at an air-solid interface. Starved liquid culture cells undergo changes observed in aggregating cells and will aggregate after a reduced time if deposited on a solid surface, but the precise relation of this differentiation to differentiation in normal development remains in some doubt. Unfortunately, no quantitative measurements are available for PD in aggregating cells.

This investigation provides a glimpse of the insight into functioning of developmental control systems which may result from attempting to relate molecular to morphogenetic parameters in a suitable experimental system. It is clear that full understanding of the *D. discoideum* signalling system and of the role of the PD in its functioning will depend on measurement of the other signal and response parameters and investigation of their interactions, but Gerisch *et al.*'s investigations are an exciting step in the right direction.—From a correspondent.

CHEMICAL CARCINOGENS

Mutagenic Potential

from our Cell Biology Correspondent

MALIGNANT transformation induced by chemical carcinogens is stable; once a cell has been transformed its progeny are transformed. The most obvious way to account for chemical carcinogenesis is to suggest that it results from chemically induced mutations, in which case potent carcinogens should be potent mutagens. When, however, carcinogens such as the polycyclic hydrocarbons were tested for mutagenic activity in conventional assay systems, involving the use of bacteria or phage as target organisms, they were found not to be strongly mutagenic. Such observations cast something of a shadow over the somatic mutation theory of chemical carcinogenesis. Chiefly as a result of the recent work of Heidelberger and his associates, however, it is now clear that polycyclic hydrocarbons *per se* are neither potent mutagens nor potent carcinogens, but epoxides produced during the intracellular metabolism of the parental hydrocarbons are. The failure to detect mutagenic activity of classical carcinogens in microbial assay systems apparently stemmed from the failure of these organisms to metabolize the carcinogens and so activate them.

As long ago as 1950 Boyland postulated that epoxides are produced during the metabolism of polycyclic hydrocarbons. In the past 18 months Heidelberger's group and others have detected epoxides as intermediates of the metabolism of these compounds in microsomes. Heidelberger's group have also established that these epoxides react with DNA and protein and that they are more potent carcinogens than the parental hydrocarbons. Huberman, Aspiras, Heidelberger, Grover and Sims (*Proc. US Nat. Acad. Sci.*, **68**, 3195; 1971) can now report that the epoxides produced during the metabolism of methylcholanthrenes and benzanthracenes are mutagenic as well as cytotoxic for Chinese hamster cells in culture; the epoxides of 7-methylbenz(a)anthracene, for example, produced 5 per cent 8-azaguanine resistant mutations among cells which survived exposure to the epoxide. Some phenols derived from polycyclic hydrocarbons are also more mutagenic and more cytotoxic than the parental compounds.

In spite of differences in the response of different sorts of cells to the various epoxides and phenols, Huberman *et al.* confidently conclude that the polycyclic hydrocarbons, which themselves are of low chemical activity, require to be metabolically activated before they become potent mutagens and carcinogens;

moreover, the epoxides are most probably the ultimate carcinogenic and mutagenic metabolic derivatives of these compounds. It is noteworthy that Cookson, Sims and Grover reported in December (*Nature New Biology*, **234**, 186; 1971) that several epoxides of the methylcholanthrenes and dibenzanthracenes are mutagenic for phage T2, the mutagenic potential of these epoxides correlating positively with their carcinogenic potential. All in all, therefore, it seems that at least some chemical carcinogens are carcinogenic because they are able to mutate their target cells.

Of course, if transformation by the polycyclic hydrocarbons depends on the metabolic activation of the parental compounds, cells pretreated in such a way as to increase the activity of the microsomal enzymes involved in this metabolism might be expected to be more sensitive to transformation by a hydrocarbon and perhaps less susceptible to its cytotoxic effect. Dipaulo, Donovan and Nelson (*Proc. US Nat. Acad. Sci.*, **68**, 2958; 1971) report examples of just such an enhancement of transformation and protection from cytotoxicity. For example, Syrian hamster cells pretreated with the weak carcinogen benz(a)anthracene are subsequently more susceptible to transformation by the potent carcinogens benzo(a)pyrene or 3-methylcholanthrene and are less susceptible to their cytotoxicity than are cells not pretreated. Such findings indicate not only that the carcinogenic and cytotoxic properties of these carcinogens can be dissociated but also that the former property depends on metabolic activation.

MEDICAL RESEARCH

Dialogue on Cancer

from a Correspondent

THERE has been much argument recently among the cancer research fraternities as to the correct balance between applied, clinically orientated studies, and those more fundamental operations which seek answers to relevant basic biological problems. As a contribution to the discussions, Dr H. E. M. Kay, dean of the Institute of Cancer Research in London, thought it would be useful to continue and enlarge upon previous teaching at the institute by a course in the principles of clinical and experimental oncology. The idea was to ask distinguished protagonists of both the clinical and experimental views to discuss, perhaps appropriately in the British Museum (Natural History), on specific topics, discussion of which might be fruitful both for the institute and for those other members of the scientific community who could afford

to come along (£2 per head per meeting for non-institute members). It was agreed that these occasions should be on Monday evenings at 5 pm and should last two or more hours. Vigorous discussion was envisaged. The attempt to create a dialogue was at least praiseworthy and accords well with the dictum *Medice, cura te-ipsam*.

The series of twelve meetings began on January 10, and in the unenviable starting position Dr R. J. C. Harris (Microbiological Research Establishment) enacted his brief which was to survey significant aspects of cancer research. He pointed out that the viewpoint of the experimentalist was often restricted in relation to the variability of malignancies. The clinician, on the other hand, relied essentially on histopathological diagnoses for the formation of his attitudes. Various characteristics of malignant cells such as loss of contact inhibition, neoantigens, alteration of glycolytic pattern, cell surface changes (which affect agglutinability by various plant lectins) and enzyme peculiarities have been adduced but it was usually difficult to decide which, if any, of these phenomena were relevant to the malignant change *per se* and which were consequent upon it. Dr Harris pointed out that poor understanding of normal differentiative processes put some limit on the interpretation of the sort of changes which can occur at the onset of malignant conditions.

Many of the salient features of cancer research such as carcinogenesis by azo-dyes, soot and viruses had been discovered many years ago but perhaps much of the early work, particularly on experimental animals, had been restricted by the failure to use inbred animals, first, to transplant tumours with success and, second, to obtain reproducible results.

Dr Harris felt that the study of tumours in domestic animals was particularly important because these animals share the human environment to a considerable degree. He quoted various of the relevant statistics and some of their pitfalls. For example, the incidence of testicular tumours was higher in dogs than cats, but this may reflect the lower incidence of testes in man's feline friends rather than anything else.

It has been established that most if not all carcinogens are mutagenic but this did not necessarily mean that all mutagens are carcinogens. With these and other examples Harris briskly breathed on and polished anew some of the well loved chestnuts of cancer research workers.

Sir Richard Doll (University of Oxford) tackled the problem of the age dependency of cancer. By urging the audience at speed over the various slopes and peaks of graphs relating tumour incidence to time, Sir Richard

made the points that age *per se* is probably not a significant feature of the causality of many cancers; increasing age could simply entail longer exposure to the same carcinogenic agencies. The second conclusion he derived was that where progressive increases in cancer incidence could be recorded with increasing age the pattern is very much dependent on specific cancers (and by inference causal factors) and could not readily be generalized. Sir Richard showed how a relatively simple exponential increase formula could be used to establish straight line predictions of tumour incidence and that the functions regulating incidence could be established in those instances in which expectation and observation coincided.

The discussion was brief and hinged largely on Sir Richard's provocative presentation which, when pressed, he allowed did not give much information in relation to possible control mechanisms, but did show that they probably did not change with age.

HEPATITIS

Infection in Hospitals

from our Medical Virology Correspondent

OUTBREAKS of viral hepatitis among patients and members of the staff of maintenance haemodialysis and renal transplantation units seem to be inevitable and the incidence of infection continues to increase. Many reports since 1965 have indicated that the infection is usually serum hepatitis (long-incubation type B viral hepatitis), which is presumably introduced by blood transfusions and subsequently spread by extracorporeal circulation used in haemodialysis. The oral and aerosol routes seem to be real modes of transmission, though are presumably uncommon.

During the period 1964–1966 an outbreak involving eighty cases of hepatitis occurred in Stockholm hospitals carrying out haemodialysis and kidney transplantation. In 1966, there were three deaths in an outbreak of hepatitis at the Manchester Royal Infirmary. In the Liverpool Regional Urological Centre, two nurses and two technicians developed hepatitis in March 1966: by January 1967, fourteen nurses, three technicians and five patients contracted the infection, and by the end of 1970 a total of fifty-five cases occurred in the unit, the associated laboratories and among relatives of patients. There were seven cases of hepatitis in six of the eleven families of patients who were dialysed at home. A recent report from Stockholm states that 11 per cent of staff members at risk developed clinical hepatitis and the

average duration of absence from work was 102 days (L. G. Collste and associates, *Scand. J. Infect. Dis.*, **3**, 113; 1971).

The experience in the United States of America is no different. Reports for the period 1966–1970 reveal that hepatitis occurred in fifty-two of sixty-five (80 per cent) of the dialysis units (*NCDC Hepatitis Surveillance Report*, No. 33; 1971). Three of the most recent and serious outbreaks of hepatitis occurred in Edinburgh, with eleven deaths including four members of the medical staff; at Guy's Hospital, London, where sixty-nine cases of hepatitis had occurred, including thirty-two members of the staff (*Brit. Med. J.*, **4**, 255; 1970) and in Sweden (E. Nordenfelt *et al.*, *Acta Path. Microbiol. Scand.*, Sec. B, **78**, 692; 1970), where thirty members of the staff and thirty patients contracted Australia antigen-associated hepatitis, as well as a number of relatives of patients and staff.

The experience in Europe and in the United States suggests that the course of illness in medical staff is more severe but also shorter in duration than in patients, and the Australia antigen is detected in the serum in this group for only a brief period. In contrast, the course of hepatitis in patients with chronic renal disease is mild or sub-clinical, but Australia antigen tends to

persist in the serum for months or years. These differences in the host response to infection are probably related to the depressed immunological response to patients with chronic renal failure.

Treatment of patients by maintenance dialysis involves regular and repeated contact with their blood. The difficulties associated with disinfection and sterilization of the equipment and the aseptic techniques which are practised in many units would not preclude the spread of a blood-borne agent. Several patients are usually maintained by one machine. Furthermore, it is now accepted that serum hepatitis may be spread by direct non-parenteral contact.

The availability of tests for detecting Australia antigen, a specific marker of serum hepatitis, provides the means of excluding at least a proportion of blood containing the serum hepatitis virus. Regular screening for Australia antigen and antibody of all patients and staff will help detect new cases and allow them to be isolated. The provision of disposable dialysers is desirable, and there is also need to reduce the number of blood transfusions. The introduction of strict methods of controlling cross-infection in the unit and associated laboratories is of paramount importance.

Hepatitis outbreaks in artificial kid-

Strongly Coupled Diffusion Fluxes

MULTI-COMPONENT diffusion has always been a subject in which it is difficult to see the wood for the trees. The theory is bedevilled by the large number of different conventions available for defining relative velocities, fluxes and so on, and in the many important practical applications the diffusion takes place through a natural or artificial polymer membrane of uncertain properties. In experiments on free diffusion, coupling between the diffusions was always found to be small, although not negligible where the verification of Onsager's reciprocal relations is concerned. Although strong coupling is more familiar in biological systems, the explanation in this context has usually introduced *ad hoc* membrane parameters. Thus the prediction, *a priori*, of large specifically diffusional effects requires some direct physical insight; this has now been supplied by Cussler and Breuer of Unilever in a study to be published in next Monday's *Nature Physical Science* (January 24).

Instead of the usual ternary system of two solutes in one solvent, Cussler and Breuer consider a salt X in a mixed solvent $A+B$. Crudely, their reasoning is that if X is much more soluble in A than in B it will be in a hurry to travel

from a region rich in B to one poor in B . As these authors point out, this is well known formally—the presence of a concentration gradient of B contributes to the flux of X a term proportional to the gradient and to the rate at which the chemical potential of X , dissolved in A , varies as B is added. But what has not been remarked before is that when B is a solvent this rate of change can be estimated very simply from the difference in solubility of X between A and B , and that this can be very large indeed. For example, among the experiments reported, the addition of acetone at aqueous Na_2SO_4 can increase the flux of salt by an order of magnitude. If the concentration gradient of B is in the opposite direction to that of the salt, the opposite effect is predicted; the flux of salt should be decreased and even reversed, so that a weak solution may be used to strengthen a strong one.

These effects will obviously have to be considered when explaining transport processes across cell membranes, and may be of considerable practical use in inducing diffusion—as in dyeing of fibres, or in formulating pharmaceutical or cosmetic skin preparations; Cussler and Breuer have already made a start on antibiotics and other systems.

ney and renal transplantation units have a serious effect on the morale of members of the unit, the laboratories and the hospital. The repercussions inevitably lead to problems in clinical management, recruitment of staff, administrative and financial difficulties. Indeed, hepatitis affects the whole future of renal dialysis and transplant programmes. The results of the deliberations of Lord Rosenheim's committee, appointed by the Department of Health and Social Security to consider these vexed problems, are therefore awaited with great interest.

DEEP SEA FISH

Toothless Maturity

from our Marine Vertebrate Correspondent

THE deep sea fishes of the genus *Anotopterus* are usually described as distinctive on account of the remarkably long palatine teeth in their elongate jaws. The capture in 1961 by RRS Discovery II in Madeiran waters of an apparent *Anotopterus* which was completely toothless, as recently reported by G. E. Maul (*Bocagiana* (Museu Municipal do Funchal), No. 28, 1; 1971), is therefore of some interest. The specimen described by Maul was taken in an Isaacs-Kidd Midwater trawl, SSE of Funchal, fished at 2,500 m, although as some of the thirty-three species of fish caught at this station are near-surface living species and this net did not have a closing device, it is possible that the specimen of *Anotopterus* was caught in more shallow water than this during hauling.

Anotopterus pharao is the only known species of the genus, and it has been found widely distributed in both the Atlantic and Pacific Oceans. It is described as a long thin fish, with pointed beak-like jaws and a long head; its large teeth are distinctive, and in coloration it is dark on the back with metallic golden tints on the sides. Specimens up to 87 cm have been described. The Madeiran specimen, which measures 76 cm, is referred to *A. pharao* by Maul in spite of its lack of teeth, and slight variation in colour, because he found on dissection that it is a mature fish with enormously developed gonads. As Maul points out, although a large number of *Anotopterus* have been caught and described, this is the first sexually mature specimen to have been noticed. He concludes that in this species sexual maturity is accompanied by a complete loss of teeth, and a consequent atrophy of the gut, for in his specimen the gut is thin walled, empty and has no internal parasites.

Atrophy of the gut and degeneration

of jaws and head bones are known in other fishes. A notable example is the Atlantic salmon (*Salmo salar*) in which the head bones become decalcified as the gonads reach a state of maturity. The salmon, however, does not completely lose its dentition, but cases of reduction in the number of teeth or gill rakers are known in other deep sea inermous fishes.

Maul suggests that the causative factor of toothlessness may be a cessation of tooth replacement (which is a continuous process in *Anotopterus* and other fish species). He also discusses the possibility that it might be accounted for by vitamin D deficiency in the deeper sea interfering with the availability of lime salts, a thesis put forward by Parr (*Bull. Bingham Oceanogr. Coll.*, 3 (7), 1; 1937) to account for the frequency of poorly calcified skeletons in deep sea fishes. The view that poorly calcified fishes are necessarily deprived fishes seems unfortunate, for in the deep sea a light skeleton, and near neutral buoyancy, may be a positive advantage to the conservation of energy. Toothlessness in sexually mature *Anotopterus pharao* may be accounted for by a need to conserve basic materials for gonad

development, as well as a compensating reduction of weight in skeletal material as the gonads grow.

A curious feature in the discovery of a sexually mature *Anotopterus* off Madeira is that according to the known distribution of this species in the northern hemisphere the larger known specimens have been captured in high latitudes, the smaller ones nearest the equator. The inference drawn by other workers was that the species migrates regularly northwards as it grows, and Maul now proposes that a reverse migration must be made by the maturing adults to bring them back into tropical (and Madeiran) waters to spawn. This is a possibility, and might in time be confirmed by the capture of large, maturing *Anotopterus* in the area intervening between the tropics and Arctic seas. On the other hand, the evidence to date can be equally well interpreted to show that a small proportion of the *Anotopterus* population moves northwards, outside the area of normal distribution for the species, grows to a larger size but never reaches sexual maturity. As other deep sea fishes behave in this way, it would not be surprising if this did not occur also in *Anotopterus*.

Part Sequencing of Viral Messengers

In *Nature New Biology* next Wednesday (January 26) Nichols, Hay and Joklik report the sequence of the first twenty-five residues at the 5' terminal end of a class of reovirus messenger RNAs—molecules which are translated in eukaryotic cells. Perhaps the most notable feature of this sequence is the absence of any chain initiation codon; apparently at least four of the ten reovirus messenger RNAs, like the genome and messenger RNAs of the RNA bacteriophages, start with a run of bases which is not translated and which presumably functions either as a ribosome attachment site and/or provides recognition signals for RNA replicase.

Reovirus has a most curious genome; not only is it made of double stranded RNA but it also comprises ten discrete segments which are classified by size into large, medium and small classes. Each segment is transcribed into a single messenger RNA and at least eight of these ten molecules are translated as monocistronic messengers. Reovirus cores, obtained by removing the outer capsid from intact virions, transcribe *in vitro* all ten segments of the genome at a rate of about 600 bases per minute at 37° C. By cooling to 31° C and starving for ATP Nichols and his colleagues cut this rate of synthesis so that the four molecules of the small class are synthesized preferen-

tially at a rate of about 40 bases per minute. Under these conditions, by feeding suitably labelled nucleoside triphosphates, they managed to obtain a mixture of the four sorts of small reovirus messengers labelled at their 5' termini. The sequence of the first twenty-five bases was then analysed by conventional methods.

All four molecules apparently begin with the same sequence (p)ppGpCpCp-ApUpUpUpUpGpCpUp(C,U)UpCpCpApGpApCpGpUpUpGp. Nichols *et al.* make the following comments about this sequence. The lability of the 5' phosphate residue presumably reflects the presence of nucleoside triphosphatases in the viral cores; there is no initiation codon; the second base is a cytidylate residue according to Nichols *et al.* although Banerjee *et al.* (*Nature New Biology*, 230, 169; 1971) have reported a penultimate uridylylate residue; the sequence of the first nine bases of these reovirus molecules is the same as that between positions 48 to 56 of R17 and f2 RNA bacteriophage RNA.

When more of these reovirus molecules have been sequenced the significance and extent of such similarities may emerge; it will be particularly interesting to know the sequence of the reovirus molecules adjacent to the initiation site.

CELLS

Mauling the Membrane

from our Molecular Biology Correspondent

WHEREAS many antibiotics attack the biochemical vitals of the bacterial cell, others are known to interfere in one way or another with its controlled communication with the outside world. One such, whose mechanism of action has been notably obscure, is bacitracin. This is a peptide of curious structure, synthesized by some strains of a *Bacillus*. It contains, among other unusual features, a tract of residues joined in the form of a thiazoline ring. Its effects on cells are various, and all fatal. It evidently acts at both cell walls and membranes, and in the latter it induces permeability to potassium ions. It requires divalent metals for activity, and these are directly complexed by it.

One of the modes of function of bacitracin is to block the synthesis of a vital cell wall component, peptidoglycan. Stone and Strominger (*Proc. US Nat. Acad. Sci.*, **68**, 3223; 1971) have now uncovered a highly specific interaction of bacitracin with a membrane component, which seems to lie at the root of this effect. They have shown that in the presence of a divalent cation the bacitracin binds strongly to the membrane constituent, C_{55} -isoprenyl pyrophosphate, so preventing its cleavage by a pyrophosphatase, with release of the lipid carrier implicated in the cell-wall synthetic cycle. Complex formation was demonstrated by a column chromatographic method, and was found to be prevented by EDTA and other chelating agents. The authors have built models to show how the antibiotic and its target molecule might interact. The possibility exists, of course, that the bacitracin disturbs the proper functioning of membranes by direct physical distortion, as well as causing lipid depletion.

A quite different kind of molecule, which is death to membranes, though after a quite different fashion, involving apparently a cataclysmic structural disturbance of the bilayer, is the polyenic visual pigment constituent, retinal. Red cells, exposed to very low concentrations of retinal, lose their ability to retain potassium ions, and at slightly higher concentrations—still, however, in the micromolar range—haemolysis ensues. Azuma and Yoshizawa (*Biochim. Biophys. Acta*, **249**, 135; 1971) have now shown that the efficacy of the retinal in both these respects depends on its stereoisomeric configuration. Both potassium release and haemolysis occur at markedly lower concentrations of the all-*trans* isomer than the 11-*cis*. Now it is the bent 11-*cis* species that forms the prosthetic group of the visual pig-

ment, rhodopsin. It undergoes photoisomerization, followed by hydrolysis from the protein, on exposure to light, and this event is accompanied by a membrane process—reportedly the release of potassium ions from the rod outer segment—which triggers nervous excitation. Now because rhodopsin is the major protein component of the rod outer segment membranes, it is an attractive proposition that the liberated prosthetic group might itself be the agency that brings about depolarization. The greater proclivity of the isomeric form, in which the molecule normally exists after its release to penetrate, or at any rate modify, the membrane, provides new fuel for this hypothesis.

Other polyenic species are familiar as membrane-active antibiotics, and like a number of peptide antibiotics, of which the best known is perhaps gramicidin A, they function, so the speculation goes, by forming aggregates that make tunnels in the bilayer, through which potassium ions can travel. By this means the active transport system is bypassed, with dire results for the cell. This proposed mechanism stands in contrast to that of the macrocyclic antibiotics, such as nonactin and valinomycin. These have the property of sequestering sodium or potassium ions, and are thought to operate as vehicles for carrying such ions through the hydrophobic barrier of the membrane. These conjectures have been strikingly upheld by the outcome of a critical experiment by Krasne, Eisenman and Szabo (*Science*, **174**, 412; 1971).

It is known that in both real mem-

branes and artificial bilayers, a change of temperature can bring about an internal phase transition, in which the paraffin chains pass from a close-packed hexagonal lattice to an essentially disordered fluid state. Ion-carriers would then be expected to operate only in the fluid phase, whereas the transport of ions through tunnels should be substantially unaffected by the condition of the hydrocarbon milieu. These predictions are precisely borne out by the measurements of Krasne *et al.* Bilayers were prepared by surface spreading of mixtures of glycerol esters. Melting, which can be observed by simple physical criteria, occurs, within a range of about 2°, at about 40° C. The transport of ions across the bilayer was measured by conductivity, and in the presence of the potassium and the macrocyclic carriers, nonactin or valinomycin, this parameter was found to undergo a violent change, encompassing from three to four orders of magnitude, at the melting temperature. The effect is reversible. In the melted state, the conductivity is directly proportional, as it should be, to the concentration of carriers.

The conductivity mediated by gramicidin A displays altogether different behaviour. There is no sharp discontinuity on melting, and the conductivity is large in both states. Only the temperature coefficient of conductivity changes with the phase transition. This must be seen as strong support in favour of the two fundamentally different mechanisms of action of the two classes of antibiotics.

More X-rays from NP 0532

FURTHER observations of the pulsed X-ray emission originating in the Crab pulsar NP 0532 are reported in next Monday's *Nature Physical Science* (January 24) by the group at the Centre d'Etudes Nucléaires de Saclay in France, who have looked in particular at the possibility of short term variations in the characteristics of the pulses. Their data come from two balloon flights carried out from southern France, the first on September 15, 1970, and the second on September 22, 1970. On each occasion the Crab Nebula was observed for three hours by detectors sensitive to the energy range 15 keV to 150 keV.

As others have done, the French group have noticed that in the X-ray region the pulse that dominates the optical observations of the Crab pulsar in fact carries less energy than an interpulse that to the optical observers appears less significant. In the optical region the energy in the interpulse is about a half that in the main pulse, but

at 0.5–9 keV the energies are about equal. In the 15–150 keV region covered by the French group the energy in the interpulse is 1.38 times that in the main pulse. But when they divided their results into two ranges of energy—15–50 keV and 50–150 keV—they find that their data are not sufficient to trace the development of the interpulse with confidence.

They also reported the absence of evidence for significant changes with energy of the proportion of the X-ray emission from the direction of the Crab that is pulsed. According to the sum of their data, 22.16 ± 2.54 per cent of the X-rays received from the Crab by their detectors are pulsed.

It is fortunate that the two flights bracketed a sudden change in the velocity of the wisps of material in the part of the Crab Nebula adjacent to the pulsar (September 20, 1970), but no significant changes in behaviour were detected on the second flight of the balloon.

MATERIALS SCIENCE

Super-ionic Conductors

from our Materials Science Correspondent

ANXIETIES about the ill-effects of traffic fumes and the problems of maintaining oil supplies combine to keep alive interest in the potential of electric cars. Such cars require either fuel cells (currently in the doldrums) or rechargeable batteries, and these must be designed for high energy density. This condition can best be met, it currently appears, by high-temperature batteries, of which Ford's sodium-sulphur battery is the best known, and such cells need electrolytes capable of standing up to fierce chemical onslaughts at high temperatures.

The sodium-sulphur cell (which is currently being examined in a number of industrial laboratories, including British ones) makes use of a solid electrolyte, beta-alumina. This extraordinary material is a refractory which, in spite of its name, contains some sodium and approximates to the composition $\text{NaO} \cdot 11\text{Al}_2\text{O}_3$; the sodium (or any of a number of alternative metals) stabilizes the anomalous crystal structure. Beta-alumina has an extremely high conductivity, much closer to that of a metal than to that of a normal ionic conductor such as NaCl, but, unlike a metal, its conductivity rises steeply with temperature.

Because of its potentially major industrial importance, and simultaneously because it poses a fascinating scientific puzzle, beta-alumina is the object of much basic-cum-strategic-cum-applied research (*pace* Rothschild), and a good deal of this has been centred on its crystallographic structure. The most impressive piece of crystallographic work yet to be done on the compound has just been published by W. L. Roth (*J. Solid-State Chem.*, **4**, 60; 1972). Roth used small monocrystals in which he substituted silver for sodium by means of ion-exchange in a salt bath; he used this method because heavy atoms are easier to locate by X-ray diffraction. The weight change during ion-exchange served to establish accurately the sodium content of the original material, which is about 25 per cent above the $\text{NaO} \cdot 11\text{Al}_2\text{O}_3$ stoichiometry.

Starting from X-ray diffraction data, a very long and scrupulous process of structural refinement was performed, with the following principal results. All the silver is in the horizontal mirror planes (in other words, planes normal to the hexagonal axis) between the "spinel blocks" of the structure. There are two kinds of ideal silver sites within the mirror planes, at intersections with the three-fold axes of the space group: the silver atoms are statistically distributed among (and also slightly dis-

placed from) these ideal sites, the rest remaining vacant; the excess charge is probably compensated by interstitial oxygen. There is a huge anisotropy of thermal vibrations of the silver ions, the amplitude being about 1,000 times greater in the mirror plane than normal to it. The structure in the mirror planes has quasi-liquid properties, the silver ions jumping very easily from one site to an adjacent vacant one. (In fact, the r.m.s. vibrational amplitude at room temperature of the silver ions in the mirror plane is almost one-tenth of the corresponding interatomic distance which, according to Lindemann's law of melting, is an approximate criterion for the melting of a solid.) The notion that the conducting ions in compounds of the beta-alumina family move in a two-dimensional quasi-liquid environment, confined by blocks of non-conducting ions, is not new, but Roth has unusually clear evidence to substantiate it.

Finally, Roth shows that the apparent randomness of the silver distribution and the hyperstoichiometric silver content can be interpreted on the basis of a model of small domains, only 5–10 Å wide in the *a* direction, in any one of which all silver atoms are restricted to one of the two types of silver sites; the hyperstoichiometry results from the necessary bunching of silver ions in the domain boundaries.

The results require some modification before they are applied to normal (that

is, sodium-stabilized) beta-alumina, because there is evidence that the sodium-silver substitution shifts the sites of the metal ions somewhat. Nevertheless, there is now available the basis of a much closer understanding of the essentially two-dimensional super-ionic conduction of normal beta-alumina and its variants.

The new term, super-ionic conduction, has been introduced by M. J. Rice and W. L. Roth in a survey report which is to be published in the next issue of the *Journal of Solid-State Chemistry*. The term is to apply collectively to three categories of highly conducting ionic compounds: the beta-aluminas, certain silver halides and related compounds (among which RbAg_4I_5 is the most remarkable), and some "defect-stabilized ceramics", of which calcia-stabilized zirconia is the most familiar. In their article, Rice and Roth replace the standard hopping model of solid ionic conduction by the notion that the conducting ions, given a specific minimum of energy, are freed from their localized states to move as free ions with measurable mean lifetimes and mean free paths (which may be of the order of interatomic spacings or substantially greater, as in calcia-stabilized zirconia). On the basis of this metal-like behaviour of the ions, the authors are able to make predictions about the thermionic power and frequency-dependence of conductivity which allow them to substantiate the basic applicability of their model.

Assay for a Duck Globin mRNA

NEXT Wednesday in *Nature New Biology* (January 26) Pemberton, Housman, Lodish and Baglioni report that a 10S RNA fraction isolated from polyribosomes of duck erythroblasts contains at least one and probably several species of duck globin messenger RNAs. Pemberton *et al.* present direct evidence that this 10S RNA contains messenger for the α globin of one of the two species of haemoglobin (haemoglobin II) in adult duck erythrocytes and they show that this messenger is translated *in vitro* in cell free systems isolated from both duck and rabbit reticulocytes. This finding confirms those of Lingrel and his associates who have reported that preparations of 10S RNA from various species of reticulocytes programme the synthesis of globin in heterologous cell free systems.

The α globin chain of duck haemoglobin II, made *in vitro* in either a duck or a rabbit cell free system is, however, unusual; it retains its N-terminal methionine residue, presumably because the polypeptide chain assumes some unique structure which prevents the enzymatic cleavage of this terminal residue. As a result, when for example a

cell free system from either duck or rabbit reticulocytes is programmed with 10S RNA containing the α II globin messenger and supplemented with yeast ^{35}S -methionine-tRNA_F, the ^{35}S -methionine is stably incorporated at the N-terminus of the α II globin molecules that are made. This means that the incorporation of N-terminal methionine from initiator methionine transfer RNA_F provides an assay for duck α II globin messenger RNA activity in RNA preparations.

An assay for eukaryotic messenger RNA is something molecular biologists have longed hankered after, and Pemberton and his associates have, apparently, not been slow to exploit their discovery. Although they give no details, they claim to have used it to determine the relation between nuclear heterogeneous RNA and cytoplasmic RNA in duck erythroblasts, to follow the processing of duck globin messenger RNA and to measure the extent to which globin genes are reiterated in duck erythroblasts. Details of these various experiments should make extremely interesting reading when they are published.

PHARMACOGNOSY

Sorcery to Computer

from a Correspondent

PLANTS have been extremely important sources of therapeutic agents and will become increasingly more so in the future. This was the message from the joint meeting of the Phytochemical Society and the Pharmaceutical Society of Great Britain on plant constituents of pharmacological interest held at Chelsea College, London, on January 5.

Some people may have thought of pharmacognosists as witch doctors in that their methods of selection of plants for study have relied to some extent on folklore and ancient custom. In an attempt to meet this criticism, Professor N. R. Farnsworth (University of Illinois) described a computerized survey technique which has been developed to automate the selection of likely species for pharmacological screening. The method involves data retrieval by computer of all aspects of knowledge of natural products including folklore, analyses of constituents and structure-activity and screening tests. The computer is asked to provide a list of plants which have been reported to possess some of the pharmacological properties desired. A complex scoring system is set up to eliminate plants with undesirable characteristics, plants known to contain the wrong sort of constituent and plants which have received a great deal of attention previously. The resulting list of species provides a basis for a reasonably short screening program.

Although the participants at the meeting were perhaps not entirely convinced by the scheme, the evidence put forward for its success was astounding. In a program carried out at Illinois to look for a new central nervous system (CNS) depressant, the computer came up with some 475 plants with possible activity. The arbitrary scoring system reduced this list to a mere twenty species, fourteen of which have been investigated at the present time. Even as a crude 5 per cent solution in water, the active principles of at least two of these plants possess CNS depressant activity which is greater than twenty times the activity of pentobarbitone: these particular species have received only scant scientific attention to date. It seems that at least seventy types of biological activity can be coded for using this method, and an important future is predicted for this type of work: future results will be noted with great interest.

The meeting heard a variety of other contributions which ranged from a discussion of the chemistry, pharmacology and structure-activity correlations of hashish constituents by Professor F. Korte (University of Bonn), to summaries of the chemotaxonomy and pharmacology of the solanaceous

alkaloids by Dr W. C. Evans (University of Nottingham) and of the mitragyna alkaloids by Professor E. J. Shellard (Chelsea College, London).

Perhaps the most exciting area of current research was that reported by Dr K. Jewers (Tropical Products Institute, London) concerning the study of a whole spectrum of biological activity shown by lactone constituents of various tropical plants. Studies *in vivo* in mice of extracts from several species of *Goniothalamus* growing in peat swamps of Sarawak have revealed the presence of a compound which displays CNS activity. The active principle, goniothalamine, has been isolated and it has been shown to be structurally related to kawain (an active constituent of the CNS active drug kava, an interesting account of which was given by Professor R. Hansel of the Free University, Berlin). An important property of goniothalamine is its marked antifungal activity (minimum inhibitory concentration 7–30 $\mu\text{g/ml.}$), and research is at present in hand on the preparation of derivatives of this α -pyrone for further pharmacological screening. A second important aspect involved pharmacological studies on limonoid bitter principles. Recent screening of methyl angolensate and products of its thermal degradation indicates interesting analgesic activity worthy of much further research.

Reviewing recent developments in antibiotic research, Mr B. Lynn (Beecham Research Laboratories) emphasized that major advances in the next few years are likely to proceed from structural modification of existing agents rather than by the introduction of completely novel compounds.

Blast Waves and the Galaxy

THE discovery in 1957 that there is a flow of gas outwards from the centre of the galaxy and the increasingly compelling evidence of violent occurrences in galactic nuclei has stimulated several studies of the kinematics of interstellar gas. The basic phenomena that have to be taken into account are the existence of a central disk-shaped galactic nucleus, of radius about 800 pc, which rotates rapidly but does not expand appreciably, the expanding arm—a relatively dense region of neutral hydrogen about 3 kpc from the galactic centre—which has an outward velocity of 53 km s^{-1} superimposed on the galactic rotation, and the irregular distribution of less dense gas between the nucleus and 3 kpc which has outward velocities of up to 200 km s^{-1} .

A further contribution to the discussion is made in next Monday's *Nature Physical Science* (January 24) by L. P. Rossner of Brown University, who points out that a blast wave originating from an explosion at the

COSMOLOGY

Another QSO-Galaxy Pair

by our Cosmology Correspondent

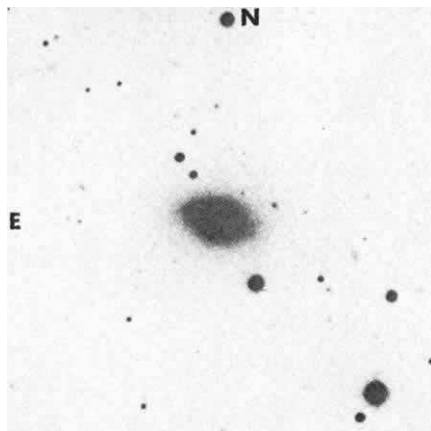
MORE evidence of a possible association between galaxies and quasars, and another blow to the cosmological interpretation of quasar redshifts, has been provided by the identification of the optical object associated with the radio source of 3C 455 as a QSO. This object lies just 23" away from the galaxy NGC 7413, which was first identified as the optical counterpart of 3C 455. But the redshift of the QSO has been measured as 0.543, whereas the light from NGC 7413 is shifted by only $z = 0.0332$ (Arp, E. M. Burbidge, Mackay, and Strittmatter, *Astrophys. J. Lett.*, **171**, L41; 1972).

The SO galaxy NGC 7413 was suggested as a likely candidate for the radio source in 1965. Improved radio positions showed, however, that the galaxy lies roughly 20" south-west of the radio position. It is now clear that the radio position coincides with a blue object (Elsmore and Mackay, *Mon. Not. Roy. Astron. Soc.*, **146**, 361; 1969). Arp *et al.* have studied plates and spectra of this object made with the Hale 200-inch and the Lick 120-inch telescopes. The blue object appears stellar, with a magnitude between 19.5 and 20, and although there is no visible connexion between this object—now identified as a QSO because of its appearance—and the galaxy, there is photographic evidence that NGC 7413 is asymmetric, and that the luminous matter forming its major axis extends into the quadrant adjacent to the QSO.

By itself this observation could not

galactic centre (the basis of one suggested explanation of the outward gas flow—see, for example, J. H. Oort, *Trans. Internat. Astron. Union*, **XIII**, 789; 1964) would be modified because of the gravitational acceleration towards the centre and because of the conservation of angular momentum of the material propelled by the wave. He finds that as a result of this modification some of the material carried along by the blast would fall back towards the galactic centre.

The behaviour of the velocity and pressure profiles with time, as predicted by Rossner, indicates the formation of a "tail shock" behind the shock proper. As the forces acting on the gas are "equivalent to tying down each fluid element to its initial position by a non-linear spring", Rossner therefore concludes that the conditions necessary for the formation of a central condensation (the nuclear disk at the galactic centre) could very well have existed according to a model such as the one he considers.



Galaxy NGC 7413. The QSO identified with the radio object 3C 455 is the nearest starlike image to the north-east of the galaxy, separated from it by roughly $23''$ (from *Astrophys. J. Lett.*, **171**, plate L3; 1972).

be taken as indicating an association between the two objects, and a few years ago the great difference between their respective redshifts would have been accepted by most astronomers as conclusively against such an association. But there is now considerable evidence that quasars and galaxies are associated, and that the redshift-distance relation is not universally applicable (see, for example, Jaakkola, *Nature*, **234**, 534; 1971).

It is particularly significant in the present context that four 3C QSOs are already known to lie within a few arc seconds of bright galaxies (Burbidge *et al.*, *Astrophys. J.*, **170**, 233; 1971); even this small number of associations is unlikely to arise from a chance superposition on the sky of unrelated objects, and the discovery of another such system greatly strengthens the position of those cosmologists who argue for a real physical link between QSOs and galaxies.

PEST CONTROL

Doing without Chemicals

from a Correspondent

FROM January 4 to 7, members of the British Ecological Society, the Association of Applied Biologists and of the Pesticide Group of the Society of Chemical Industry met in Oxford for a symposium entitled "Increasing the Biological Contribution to the Control of Pests and Diseases". Although ecologists and even applied biologists might be expected to hold the point of view implicit in this title—that increasing the biological control of pests is desirable—it is encouraging to find industrial chemists, who have a vested interest in chemical control, and who could therefore look on the replacement of chemical pesticides by other means as a threat to their livelihood,

also participating in this symposium.

It was soon clear that the term "biological control" meant different things to different speakers. Some spoke of using parasites and predators to control insect pests, some discussed methods of husbandry which reduced infestation. Sterilization and genetic engineering were also advocated. The only thing in common was the negative implication that biological control meant not relying solely on chemical pesticides, whether insecticides, fungicides, nematicides or herbicides, to control crop damage, or to prevent the spread of insect-borne diseases. It was also apparent that few of the speakers foresaw any likelihood of the total abandonment of chemical controls, at least in the near future, though many expressed the hope that safer chemicals would be used more scientifically.

On the first day a series of contributions attempted to provide the ecological background to the symposium. Thus, Dr J. P. Dempster (Monks Wood Experimental Station) and Mr T. H. Coaker (University of Cambridge) described field experiments with different spacing of brassica crops, and using various types of undersowing, on the levels of pest insects and their enemies. Professor G. C. Varley (University of Oxford) discussed his classical observations to determine the key factors controlling insect numbers in an oak wood. Though this type of work already illuminates the process of

pest control, it has at present little application to agricultural problems.

The keynote address was then given by Dr F. Wilson (CSIRO, Australia) who drew on his unrivalled personal experience of the use of parasitic and predatory insects to control pest species. It was revealing, however, that he still found it useful to quote such long-established successes of biological control as that of the cottony-cushion scale in California by the vedalia beetle (1888), of the prickly pear by the caterpillar of *Cactoblastis* in Australia (1925) and of the coconut moth in Fiji by the tachinid parasite (1925). He made it clear, however, that even if these successes had seldom been equalled by later workers, real progress continued in many areas, continental as well as islands, in spite of the tiny financial investment in this type of work.

"Classical" biological control, that is the use of parasites and predators, has been useful in agriculture, but has never been effective against the vector of a human disease. Other non-chemical techniques, however, are showing promise. Dr G. Davidson (London School of Hygiene and Tropical Medicine) discussed such topics as genetical control, and the introduction of individuals with cytoplasmic incompatibilities which may give rise to widespread sterility in mosquitoes. It is hoped that such techniques will be useful in areas where disease vectors have become resistant to insecticides.

New Ways with Gel Chromatography

THE theory and practice of transport processes, particularly ultracentrifugation and gel column chromatography, applied to the analysis of dissociating macromolecular systems, have been worked out largely by Gilbert and his associates. This approach is proving of increasing value for the measurement of subunit equilibria in proteins, these being of direct relevance to cooperative interactions.

In next Wednesday's *Nature New Biology* (January 26) Gilbert *et al.* describe an interesting modification of the method, to allow a direct quantitative comparison between the equilibrium constants for two systems in dissociation equilibrium. The analysis has been applied to met- and oxyhaemoglobin. The procedure consists in equilibrating a gel column with one of the components, such that the concentration in the effluent is constant, and then switching to the other component at the identical concentration. By monitoring the effluent at an isosbestic point for the two derivatives, the elution profile can be determined. If now the position of the equilibrium (dimer-tetramer in the case of haemoglobin) is the same in both species, the concentration plateau will

remain. If it is not, there will be a perturbation, the shape of which is determined by the dimer and tetramer elution volumes and the ratio of the dissociation constants. For the system in question this ratio is not in fact unity, and by a least-squares fit to the observed profile it can be determined with excellent precision. The corresponding free energies of dissociation for oxy- and methaemoglobin differ by about 1.1 kcal mole⁻¹.

An extension of the same approach can be used to determine the relative equilibrium constants for the formation of hybrids, that is to say tetramers containing two oxyhaemoglobin and two methaemoglobin subunits. This equilibrium can be observed by first equilibrating the column with oxyhaemoglobin, and following this with a mixture of both derivatives at the same total concentration. The equilibrium constant for heterotetramer formation turns out to be almost exactly the geometric mean of those for the dissociation of oxyhaemoglobin and of methaemoglobin, from which it follows that essentially complete hybridization is to be expected. This is in accord with early observations on the species present after partial oxidation of oxyhaemoglobin.

Several speakers discussed the production of new insecticides and other chemicals which are compatible with biological control measures. Dr J. M. Cherrett (University College of North Wales, Bangor) described work on toxic baits, and the studies of insect behaviour to try to ensure that the baits are eaten. Dr C. N. E. Ruscoe (ICI Ltd, Jealott's Hill) spoke on the possible use of insect hormones or of synthetic chemicals with similar properties as insecticides; this work is still in the "basic" stage, and the audience was interested to find a commercial organization so deeply involved. Dr D. Price Jones, also of ICI, accepted the ideal that more selective insecticides are required, and described work aimed at their production, but at the same time drawing attention to commercial and other difficulties. As the last speaker in the session on principles, Professor M. J. Way (Imperial College, London) spoke about "integrated control". He showed how the natural mechanisms which may reduce damage may continue and may even be encouraged, while at the same time insecticides may be used in the way first advocated by the late W. E. Ripper and by A. W. A. Brown, "as a stiletto, not as a scythe".

The third day was devoted to accounts of practical applications of biological control. Dr R. K. Murton (Monks Wood Experimental Station) said that few vertebrates are "absolute pests", and that attempts to eliminate them are usually wasteful and ineffective. It is better to try to exploit behavioural responses and to prevent the birds from entering crops which they may damage. Mr B. Stott (Ministry of Agriculture, Fisheries and Food) showed that the grass carp, a native of Asia, may control water weeds in Britain by grazing. As it does not breed except in captivity, unwanted spread and damage is unlikely. Dr J. Rishbeth (University of Cambridge) described a practical method, now commercially available, for introducing fungi to wood to prevent the development of disease. Nematode pests of field crops are more difficult (and more expensive) to control than insects; Dr F. G. W. Jones (Rothamsted Experimental Station) discussed the possibilities, not yet realized, for the biological control of these animals in the soil. Rotations, with the absence for some years of a susceptible crop, are still the most effective processes.

There have been remarkable successes in both integrated and biological control in Britain. Dr R. Hull (Broom's Barn Experimental Station) described how the sugar beet crop is protected, with minimal use of insecticides. This depends on the application of research, made possible by the presence of some 140 trained field officers—a condition which does not apply to many other

crops. The damage by aphids and aphid-borne viruses has been largely controlled, though larks still destroy young seedlings, spaced scientifically and grown from monogerm seeds. There is, of course, not much public enthusiasm for the control of larks, either biologically or chemically. Mr I. J. Wyatt (Glasshouse Crops Research Institute) described another success story—the remarkable system now practised commercially in which cucumbers are protected from spider mite attack. This pest is resistant to many insecticides but may be controlled by a predatory mite. The unusual factor is that it is found necessary to introduce the pest, and to allow it to build up to a level where it does little damage, before the predator is liberated.

The final session dealt with the future, stressing problems of importance in Britain. Mr R. Gair (Agricultural Development and Advisory Service, Cambridge) and Mr A. H. Strickland (MAFF, Harpenden) discussed the present and the future from the grower's viewpoint. Reduction in manpower, increasing mechanization, increases in the size of individual holdings and modern marketing methods all make efficient pest control increasingly important. Biological methods which are less than 100 per cent successful have therefore little future. Dr N. W. Hussey (Glasshouse Crops Research Institute), who himself played a leading part in the work previously

described on pest control in cucumbers, discussed future research, and the ways in which new insecticides will affect integrated control programmes. Dr W. F. Jepson (Cyanamid, London) complained that today the public is more concerned about the harm that pesticides may do than with their real benefits. He showed that, in most cases, pesticides are used with safety, and he suggested that future trends in pesticide production should make for even greater safety, if public opinion does not prevent this.

Dr K. Mellanby (Monks Wood Experimental Station) gave the conservationist's viewpoint. He admitted that some ill-informed criticism of pesticides came from those interested in conserving wildlife. Real damage to wildlife had occurred from a few persistent organochlorine insecticides, and from the careless use of other chemicals, including herbicides. Many chemicals, properly used, did not, however, harm wildlife. Conservationists had often uncritically welcomed all things which could be called "biological control". Some methods, such as the production of plants resistant to pests and pathogens, cultural methods which discouraged pests and which encouraged their natural enemies, were certainly to be welcomed. Integrated control, reducing pesticide use, was also desirable. But conservationists in Britain should not welcome the introduction of living organisms from abroad without the most stringent controls.

Nomenclature for Isolated Chloroplasts

THE chloroplast is a complex membranous organelle possessing both inner and outer membrane systems. To obtain isolated chloroplasts in which all the membranes are intact requires extremely careful and rapid separation procedures (D. A. Walker, *Methods in Enzymology*, **23**, 211; 1971). Breakage or loss of the outer membranes during isolation gives preparations which show considerable differences in their biochemical properties, and for this reason there has been confusion in the nomenclature used by workers to describe their various chloroplast preparations.

With this confusion in mind D. O. Hall, in next Wednesday's *Nature New Biology* (January 26), has attempted, by considering both morphological and functional characteristics, to classify isolated chloroplasts into six main "types" (A to F). Type A are the only preparations which can fix carbon dioxide at high rates (50–250 $\mu\text{mol/mg}$ chlorophyll/h) without addition of substrate or cofactors and seem to have a fully functional outer membrane system containing specific translocase. Chloroplasts often referred to as Class

I, which possess some of their outer membrane system and appear bright and highly reflecting under the phase contrast microscope, are called type B. Type B differ from type A in that they are permeable to NADP, ferricyanide and ADP and can only fix carbon dioxide at very low rates.

The remaining groups represent a classification of those chloroplasts which have lost their outer membranes and cannot normally fix carbon dioxide but can, to different degrees, support electron transport and photophosphorylation when cofactors are added. Type C correspond to what Spencer and Unt called Class II chloroplasts (*Aust. J. Biol. Sci.*, **18**, 197; 1965) and are essentially intact lamellar systems having little or no stroma. Osmotically broken preparations are grouped into either type D or E. They differ in that type E have their outer membranes and stroma removed by centrifugation so that these components never occur in the reaction mixture. Finally, Hall has grouped all sub-chloroplast preparations consisting of small thylakoid membrane vesicles into the category type F.

Foetal "Antigens" in Cancer

PETER ALEXANDER

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In this article, Professor Alexander discusses the role played by onco-foetal antigens.

A RESEARCH assistant of Paul Ehrlich, G. Schöne, reported in 1906 that mice which had been injected with foetal tissue acquired the capacity to reject transplants of tumour tissue which otherwise grew and killed¹. Adult tissues did not evoke this response and it was attributed to an immune reaction brought about by cells, a phenomenon for which Ehrlich coined the term "athreptic immunity". The key questions raised by this experiment, which has been repeated in some tumour systems but not in others, remain to be answered. The relationship between embryonic development and neoplasia has been a central theme of cancer research ranging from the concept formulated by Conheim nearly a century ago of embryonically misplaced "cell rests" to the modern formulation of activation of silent genes normally only expressed in the embryo. Reports² of the presence of embryonal antigens in extracts from tumours date from 1930 but this area of research has recently become extremely active. Not only does it provide most valuable leads for basic cancer research concerned with the nature of the malignant transformation but there is real hope that in the near future the estimation of certain foetal antigens in tissue fluids may prove of great value for diagnosis and for monitoring the treatment of some forms of cancer by exploit-

these occurs when immunological methods are used as a method of analysis to establish that a macromolecule found in an embryo is also present in tumour tissue. The usual procedure is to raise an antiserum of either a tumour or embryo extract in a foreign species and after suitable absorptions to use it to show that substances (operationally called antigens) are common to the embryo or the tumour but absent from adult tissues (or at least these adult tissues used for absorption of the serum). The second situation is that in which a substance present both in tumour and embryo evokes an immunological reaction in the tumour-bearing host (that is, in the case of a primary tumour there is an auto-immune reaction to the foetal antigens). Although all foetal antigens belonging to this class also meet the requirements for the foetal antigens described in the first definition above, the reverse does not apply.

There are also many similarities between foetal and cancer tissue in the patterns of transfer RNA (ref. 3) and of enzyme particularly related to carbohydrate metabolism and the profile of the different iso-enzymes such as lactic dehydrogenase^{4,5} and aldolase⁶. This phenomenon has been known for a long time (see Greenstein⁷) but it has recently been reformulated in the phrase "oncogeny is blocked ontogeny". These biochemical differences between embryo and tumour on the one hand, and adult tissues on the other, cannot, however, be put to the same use as the true onco-foetal-cancer antigens because they are usually only of a quantitative nature and are also sometimes mimicked when non-dividing and dividing adult tissues (such as normal and regenerating liver) are compared.

Table 1 Classification of Cross-reacting Antigenic Substances in Tumours and in Foetal (Embryonic) Tissues

Macromolecules present in tumours and foetus	Immunogenic in heterologous host	Immunogenic in autologous or syngeneic host	Causes cytotoxic reaction in tumour-bearing host	Suggested name
(1) But also found in some normal adult tissues	Yes	No	No	Onco-foetal associated antigen (OFA-associated)
(2) Absent from all normal adult tissue but occurs late in foetal life and induces tolerance	Yes	No	No	Onco-foetal specific hetero antigen (OFA-specific)
(3) Adult recognizes as foreign because they only occur in early foetal life or are present in a cryptic form				
(a) found in interior of tumour cell	Yes	Yes	No	Onco-foetal specific auto-antigen (OFA-auto)
(b) associated with plasma membrane of tumour cell	Yes	Yes	Yes	Onco-foetal transplantation-type antigen (OFA-transplantation)

ing the truly remarkable sensitivity—nanogram measurements are routine and at picogram levels feasible—of radioimmunoassays. There is also the much more remote prospect that better understanding of Schöne's observation may lead to a new approach to the treatment of malignant disease by immunological means.

Two Situations

The term "foetal antigens" is used loosely to describe two quite different situations in relation to cancer. The first of

Table 1 is an attempt to classify the different types of onco-foetal "antigens" (OFAs). It seems necessary to coin this phrase because the more elegant description of carcino-embryonic antigen has been pre-empted to describe one class of these compounds. The different categories of OFA can in principle be used for diagnostic purposes provided that they are released intact by the tumour and appear in readily accessible tissue fluids. To evoke an immunologically mediated host reaction which is cytotoxic to tumour cells (and which might be useful for immunotherapy) the OFAs must be of the transplantation type. In other words,

the foetal antigens must be antigenic in the tumour-bearing host and must be situated on the cell membrane of the tumour if they are to constitute a target by which immune mechanisms kill cells. A host attack directed against OFAs present in the interior of the cells (that is, an "OFA-auto") is unlikely to be cytotoxic in the living cell; internal antigens are inaccessible to antibody and cell-mediated immune factors unless such OFAs are excreted by the cell, in which case they will at some time be transiently associated with the cell membrane (fibroblasts, for example, can be killed by antibodies directed against collagen).

There are now several examples where OFAs have been detected in sera by the use of a heterologous antiserum raised against an antigen isolated from tumour tissue.

α -Foeto Protein

Abelev⁸ established in 1963 that one of the abnormal proteins synthesized by a chemically induced hepatoma in mice was antigenically identical to an α -globulin present in embryonic and neonatal mouse serum but absent from the adult mouse. Within one year a similar material had been found by Tatarinov⁹ in the serum of patients with primary hepatoma. Recently¹⁰ a radioimmunoassay has been developed for α -foeto protein and, using this method, very low levels (~ 5 to 10 ng ml⁻¹) which are quite undetectable by gel diffusion have also been found in the serum from normal subjects. The level found in hepatoma patients is 1 to 100 mg ml⁻¹.

This protein, synthesized by perivascular parenchymal cells of foetal liver, has not yet been fully characterized but is thought to have a molecular weight of the order of 70,000; its existence was first detected in 1957 in the human foetus by Bergstrand *et al.*¹¹ The only human neoplasm, apart from primary hepatoma, which produces this protein in amounts detectable by gel diffusion is the primitive gonadal tumour, teratoblastoma. In a summary of the literature Stillman and Zamcheck¹² found that out of 5,301 sera studied only two gave a positive reading when there was no proven hepatoma or teratoblastoma; both these patients were very sick, however, and they died soon afterwards; no post-mortem was performed. Although the rate of false positives is, therefore, very close to zero, the false negatives are much more puzzling. In some areas of Africa 80% of all patients with hepatomas give a positive test, whereas in Western countries rates as low as 30% have been reported. Whether a hepatoma produces α -foeto protein may depend on its mode of causation, thus 100% of hepatomas induced in rats with a carcinogenic azo dye gave a positive serum whereas none did so with hepatoma caused by aflatoxin B (ref. 13).

Another protein, called α_2 H ferroprotein and related to ferritin, is also produced in foetal liver (and not found in normal children older than two months) and appears in the sera of 81% of young children with a large spectrum of tumours not confined to those of embryonal origin¹⁴. Neither of these α -foeto globulins induces an immune reaction in the host and falls either into the category "OFA-specific" or in view of the finding that very small amounts are present in normal serum it may have to be called "OFA-associated".

Carcino-embryonic Antigen

The term carcino-embryonic antigen (CEA) was used by Gold and Freedman¹⁵ in 1965 to describe a substance (or group of substances with a common antigenic determinant) detected by the use of heterologous antiserum in all adenocarcinomas of the human digestive tract but in no benign tumours. Because the antiserum was absorbed with normal colon, CEA must be absent or present only in very small amounts in the normal adult tissue. CEA was, however, detected in the digestive organs (but not in the lung)

of human fetuses aged between 2 and 6 months. The intimate—almost obligate—association of CEA with colonic tumours is demonstrated by the remarkable finding¹⁶ that this substance is produced in large amounts by a human colonic tumour adapted to grow in hamsters and propagated in the foreign host for many years. Considerable progress has been achieved in the chemical characterization of CEA; it is a relatively stable glycoprotein with a molecular weight of 1 to 2×10^5 and a procedure for purification has been described that gives a product which appears homogenous according to several physico-chemical criteria¹⁷. It is not yet clear, however, whether CEA is a single protein made only by malignant and foetal cells or whether there is a group of macromolecules with a specific antigenic determinant conferred on it by a transferase linking carbohydrates to proteins. The particular antigenic site on the CEA molecule (or molecules) on which the test depends is only accessible to the antiserum when the glycoprotein is in an extended configuration¹⁸. At physiological ionic strengths these molecules become coiled and no longer react with the antiserum; yet in the rabbit or the goat in which the antisera are raised the "coiled" material must have been antigenic. It has been suggested¹⁹ that CEAs from different tumours may have different requirements for rendering the antigenic site available for interaction with antibody in the radioimmunoassay. The resolution of this point and problems associated with the chemical identification of CEA and the nature of the tumour specificity is difficult because the glycoproteins involved are unstable, and readily aggregate and disaggregate. Immunofluorescence on frozen sections indicates that CEA is localized on the tumour or embryo cell surface; it is probably not a part of the cell membrane itself but is an extracellular material forming part of the so-called glycocalyx²⁰. The final assembly of CEA possibly takes place outside the cell.

These studies by the Montreal group of Gold, Freedman and their colleagues constitute a landmark in cancer research in linking cancer to embryonic development, not least because the work was done with human tumours. In 1969 this group made a further advance which holds the promise of important practical application²¹. They found that a CEA-like substance can be detected in the serum of cancer patients by using a radioimmunoassay. In a survey involving the sera of 200 patients an antigen was detected in thirty-five out of thirty-six patients with proven colonic or rectal tumour antigen which cross-reacted with CEA. It is not yet known whether the circulating antigen is chemically identical to the CEA extracted from colonic tumours. From a practical point of view the important aspect is that the circulating factor reacts specifically with an antiserum to CEA. In some instances detection of "circulating CEA" was the first evidence of digestive tract cancer and preceded conventional diagnosis. Moreover, the CEA test provided information on whether significant amounts of tumour had been left after treatment, and reappearance of CEA following surgery was a sure sign of recurrence of tumour growth.

In January 1971, not only did confirmation come from the Department of Medicine at Harvard of the presence of CEA in the serum of nearly all patients with carcinomas of the colon and pancreas²², but two new findings were also made. Some patients with extreme metabolic disturbances produced either by advanced alcoholic liver disease or kidney disorders gave positive CEA tests. Furthermore, CEA was found also in the serum of patients with lung tumours; this was contrary to the Montreal²¹ work in which CEA was only detected in tumours of the gastrointestinal tract. This discrepancy is all the more puzzling because the techniques used were apparently similar. The Boston experience has been repeated in a large study of patients in New York¹⁹. Apart from a very high proportion of positive tests for gastrointestinal tract tumours, CEA was found in

a significant proportion of patients having other carcinomas, notably of the lung and breast. In this investigation a different technique, developed by Hansen *et al.*¹⁸, was used for the radioimmunoassay but the antigen was prepared from a colonic tumour in the same way as in the original work at Montreal; a member of the Montreal group is in fact a co-author of this latest paper. There are two possible explanations of the discrepancy in the clinical studies; first, that the CEA-type material in the serum of patients with bronchogenic and mammary tumours differs in some way from the CEA from gastrointestinal tract tumours so that the exact Montreal procedure only picks up the latter while slight modification such as variations in ionic strength allows the detection of all types of CEA; second, that the CEAs released by different tumours are indistinguishable (that is, with regard to site specificity the Montreal study was in error). Further work is needed to distinguish between these explanations.

Another question that remains to be answered concerns the antigenicity of CEA in the patient. Using a haemagglutination technique Gold *et al.*²⁰ found antibodies to an extract from colonic tumours in 70% of patients with colonic tumour. This test was done, however, before the separation of CEA had been developed and the auto-antibodies might have been directed at a constituent of the colonic tumours other than CEA²³. The supposition that CEA is not antigenic in man is supported by the finding that intradermal inoculations of crude extracts from colonic tumours but not of purified CEA give delayed hypersensitivity reactions in patients with colonic cancer²¹. Also lymphocytes from patients with gastric cancer do not transform when cultured with CEA²⁴. At present, therefore, it is not clear whether CEA should be classified as "OFA-specific" or "OFA-auto". Indeed CEA may have to be described as "OFA-associated" because very small quantities of CEA have been detected even in normal colon by radioimmunoassay which is much more sensitive than the gel diffusion technique used originally.

Foetal Sulphoglyco Protein

A sulphoglyco protein present in foetal gut and with blood group substance A activity has been found in the gastric juice of nearly all patients with carcinoma of the stomach; but it is also found occasionally in the absence of cancer both in the gastric juices as well as the mucosa²⁵. This substance would appear to belong to the category "OFA-associated".

Using immunofluorescence and a heterologous antiserum raised against microsomes of human colonic mucosa, Nairn and his colleagues^{26,27} showed that one intestinal component disappears soon after birth but emerges again in senescence as well as in gastric neoplasia, both benign and malignant. On the other hand, a "gastric antigen" which is normally present in adult life disappears in conditions in which the "intestinal antigen" is present. The "intestinal antigen" may conceivably be related to the foetal sulphoglyco protein.

Regan Iso-enzyme

Foetal alkaline phosphatase (the "Regan iso-enzyme") is characterized by high heat stability and was isolated from a bronchiocarcinoma of a man called Regan; it is found in the placenta and serum of pregnant women but not in any normal adult tissues. In a large survey it was found to be present in the sera of some 4% of patients with a wide variety of different carcinomas and there is good evidence that it is produced by the tumour. This enzyme has provided a reliable guide for either progression or regression of the tumour following treatment^{28,29}.

OFA in Tumour Extracts

A substance with the electrophoretic mobility of a γ -globulin, and chemically and serologically quite distinct from both α -foeto protein and CEA, has been identified in saline extracts from a wide variety of human tumours both malignant and benign but not from normal tissue³⁰. Antigenically cross-reacting material was found in the serum of foetuses of many species (for example, human, bovine, and feline). This OFA was detected because it was precipitated by an antibody found in the serum of a cancer patient. Antibodies to this OFA occur only very rarely, however; they were found in eight out of 1,030 sera from cancer patients. A related OFA may exist in human leukaemia cells but the test here relies on cross-reaction between extracts from foetal tissues with a heterologous antiserum raised against a relatively purified minor constituent(s) of leukaemia cells (D. C. Viza, private communication).

Saline extracts from a large number of different mouse tumours contain an antigen which precipitated with antiserum raised against a nine-day-old mouse embryo extract³¹. The antibody was not absorbed by adult heart, lung, spleen, liver or kidney but was absorbed by adult skin. The material studied would therefore seem to belong to the category "OFA-associated".

It is probably a question of semantics whether the group specific antigen of the murine leukaemia and sarcoma viruses should be classified as an OFA. Using a sensitive complement fixation test Huebner *et al.*³² found this antigen in the embryo of all strains of mice tested as well as in many tumours. It is generally absent in young adult mice but may reappear with increasing age. According to the terminology adopted in this review this is "OFA-associated" but Huebner considers it to be the "oncogene".

Wang³³ found that saline extracts from rat sarcomas that had been induced with the carcinogen 3,4-benzpyrene produce a typical delayed hypersensitivity reaction when injected into rats immunized against such tumours. The very nature of the test used shows that the antigen detected is immunogenic in the rat. Rats in which a primary tumour had been induced—and then surgically removed—responded to the same extent to an extract from the autologous tumour as to extracts from other rat sarcomas. Extracts made from rat embryos, but not from non-malignant adult tissue, also cause delayed hypersensitivity reactions in rats immunized against sarcomas and the material responsible can therefore be considered an OFA³⁴. This OFA does not, however, seem to be related to the tumour-specific transplantation-type antigens which are present in the plasma membrane of chemically-induced sarcomas because the latter do not cross-react and seem to be unique for each individual tumour. Moreover, immunity to a chemically-induced sarcoma can only be evoked by the specific sarcoma used in the test, and this indicates that an immune reaction against the OFA (which has been found in all of the rat sarcomas studied) is not capable of restraining tumour growth. The simplest interpretation is that, although the OFA is antigenic in the adult, it is not exposed on the surface of the sarcoma cell and therefore does not provide a target for an immunologically-mediated cytotoxic reaction—that is, it is an "OFA-auto".

OFA on Cell Surfaces

There is now convincing evidence that transformation of mouse and hamster cells by the oncogenic DNA virus SV40 leads to the appearance on the cell membrane of a compound which is normally only present on early embryonic cells³⁵⁻³⁸. A heterologous antiserum raised against unfertilized mouse eggs, for example, was cytotoxic to SV40

transformed mouse cells³⁸. This OFA may not seem to be immunogenic in mice because the serum from mice immunized with syngeneic SV40 transformed cells was not cytotoxic to mouse eggs; a negative is, however, difficult to interpret. But the serum of mice immunized against SV40 transformed cells contains antibodies directed against a tumour specific surface antigen which appears in response to virus infection and against which the adult host reacts to give tumour immunity. The simplest interpretation is that virus-transformation leads to the appearance in the cell membrane of both an OFA and a new transplantation-type tumour specific antigen. The available data do not show whether this OFA is normally hidden below the surface and unmasked after a change associated with transformation or whether it is the product of a gene wholly derepressed in non-malignant adult cells. It is tempting to link these findings with changes which have been detected by other than immunological methods in the cell surface after malignant transformation and which are also shown by embryo cells³⁹. These foeto-cancer characteristics include increased susceptibility to agglutination by concanavalin A (ref. 40) and wheatgerm agglutinin⁴¹ (though not apparently an actual increase in agglutinin binding sites^{42,43}) and the appearance of specific glycolipids not detectable in adult cells^{44,45}. The changes in surface properties may be associated with the appearance of new macromolecules that are antigenic in a foreign host but not in a syngeneic host. They may have to be referred to as "OFA-associated" because the specific surface characteristics of tumour and embryo cells appear transiently in normal cells when these are in mitosis⁴⁶.

By contrast with the mouse, the common surface antigen of virus transformed and early embryonic cells in the hamster is unquestionably antigenic in the syngeneic host and embryo cells induce antibodies and cytotoxic lymphocytes directed against SV40 transformed hamster cells³⁷. Possibly there is an inherent difference in the biology of these OFAs in the mouse and hamster but variations in experimental procedures in the experiments themselves may determine whether or not a host reaction to the OFAs is produced. Coggin, Ambrose and Anderson³⁷ actually prevented the growth of a transplant of SV40 tumour in hamsters by immunization with early embryonic cells, and this is perhaps the clearest demonstration that these tumours display the transplantation characteristics of OFAs ("OFAs-transplantation"). To obtain immunity the cells had to be irradiated before implantation, otherwise, it is suggested, they mature and lose immunogenicity. Failure of others to obtain immunity in mice to polyoma induced tumours⁴⁷ and to spontaneous mammary tumours⁴⁸ with early embryonic cells could be due to the fact that the embryos were not irradiated in these experiments. A very weak immunological response directed against murine leukaemia cells has recently been reported following immunization of mice with irradiated embryo cells⁴⁹.

Some older work indicates that "OFAs-transplantation" are not confined to the SV40 tumours of hamsters but may be a more general phenomenon. Extending the findings of Schöne¹, Buttle *et al.*^{50,51} reported that inoculation of mice with embryonic liver cells completely inhibited the growth of a sarcoma implanted seven days later.

From a possible therapeutic point of view the observation made by Fibiger⁵² in 1927 seems most pertinent. He found that injection of an extract from the skin of foetal mice retarded the growth and prevented distant metastases from primary carcinoma induced by painting the skin of mice with coal tar. A similar result was reported in 1971 by Coggin *et al.*⁵³ for virus-induced tumours in the hamster. In this case the hamsters were immunized with human foetal tissue and this raises the possibility that some "OFAs-transplantation" may cross species barriers. Clearly much more research is required to elucidate if and when "OFAs-transplantation" occur and their role in tumour-host relationship.

OFAs and Tumour Specific Antigens

It has been suggested that the different transplantation-type tumour specific antigens all have a counterpart in embryonic life and that they are the products of a whole range of repressed genes which are derepressed more or less randomly during carcinogenesis by chemicals⁵⁴. Newer data are difficult to reconcile with this hypothesis and it seems more likely that a number of quite distinct changes in the surface of the cell accompany the malignant transformation. These changes include alterations in the steric configuration but not in the number of agglutinin receptors; the appearance of macromolecules (antigens) coded for by genes normally only expressed in embryo, that is, OFAs; and products of genes not expressed either in embryo or in normal adult cells. The gene responsible for antigens of the last type, that is, the true tumour specific antigens, could arise by a mutation induced perhaps by the carcinogen or it might be a gene which is normally totally silent and only expressed in the malignant state—the TL antigen is an example⁵⁵.

An OFA may not be directly related to the specific product of a derepressed gene. The actual antigenic determinant which is measured may be created from a normal cell constituent by a sugar transferring or degrading enzyme present in the tumour (or perhaps released by the tumour). It is conceivable that some OFAs may arise from the interplay of two ubiquitous cell products which are separated in the normal cell but are able to interact, intracellularly or extracellularly, in a tumour.

The presence in tumours of foetal substances (that is, OFAs of all types) may be only one aspect of a much more general phenomenon associated with malignancy, namely the synthesis of aberrant proteins of a wide variety. The production of pituitary hormones by some lung tumours and of insulin by some non-pancreatic tumours has been known for some time because the formation of these hormones, causes clinically detectable symptoms. There may, however, be many other instances of "non-physiological" gene activation in tumours of many types. It is a case of pure semantics to refer to the process whereby tumours make foetal products as dedifferentiation and to the process of making hormones as differentiation; in both cases they are an expression of an otherwise silent gene. Finally, the possibility that the switch on of silent genes is not an all or none phenomenon must be considered. The suppression of the foetal gene responsible for α -foetal protein production and possibly also CEA in normal adult tissue is remarkably efficient but not quite complete.

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Mechanisms of Interaction of a Hormone—Receptor Complex with the Genome of a Eukaryotic Target Cell

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Acidic proteins associated with the DNA of oviduct cells seem to possess tissue specificity and to contain the binding sites for progesterone.

WE have demonstrated that progesterone causes distinct changes in gene expression in the chick oviduct, followed by induction of the specific protein avidin^{1,2}. In recent studies, we have identified a progesterone binding protein in the cytoplasm of oestrogen-treated chicks ($K_d \approx 10^{-10}$ M)³. When they enter the target cell, progestins initially bind to this receptor molecule. The steroid-receptor complex is then transported into the nucleus where it becomes associated with the target chromatin⁴, after which quantitative and qualitative alterations in nuclear RNA synthesis occur, leading ultimately to the induction of synthesis of avidin^{5,6}.

Cell-free recombination studies using cytoplasm and nuclei have revealed that: (1) the oviduct receptor protein is essential for nuclear binding of progesterone; (2) oviduct nuclei but not spleen, lung, intestine or liver nuclei can bind the progesterone-receptor complex, and (3) nuclear bound progesterone can be extracted by high salt, still associated with the receptor molecule⁴. Additional studies have shown that binding of the progesterone-receptor complex to chromatin can be accomplished by direct exposure of purified chromatin to the hormone-receptor complex *in vitro*⁷. Free progesterone binds only slightly to chromatin and requires the target cell receptor for substantial specific binding. Furthermore, the hormone-receptor complex binds much better to target-cell chromatin than to other chromatins⁸. This has led us to postulate that perhaps the target cell genome contains specific "acceptor sites" which receive the inducer complex as it enters the nucleus.

Histones not Acceptor Molecules

We initially attempted to determine whether the histones were the important fraction of chromatin responsible for binding of the hormone-receptor complex to chromosomes. Selective dissociation of histone from chromatin was carried out in the presence of 2.0 M NaCl, 5.0 M urea (pH 6.0)^{8,9}. The chromatin could be reconstituted by sequential dialysis to remove first the NaCl and finally the urea¹⁰. In this way, "hybrid" chromatins could also be prepared in which histones from other tissues or species were substituted during recon-

stitution⁸. Binding of receptor to this reconstituted chromatin was similar to the intact native chromatin of oviduct. Moreover, the capacity to bind the steroid-receptor complex was retained by hybrid chromatins containing histones from a non-target tissue of a different species, for example, calf

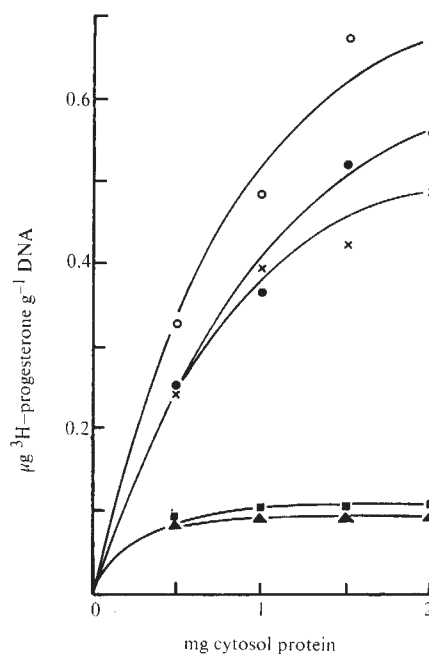


Fig. 1 Binding of ³H-progesterone preincubated with oviduct cytosol to intact and reconstituted chromatins of the chick. Tissues were removed from oestrogen-primed 20 day old chicks, minced, and homogenized in 10 volumes of 0.5 M sucrose in 0.25 M KCl, 0.002 M CaCl₂, 0.05 M Tris-HCl (pH 7.5) buffer. Chromatin was prepared from purified nuclei as described previously^{7,11}. Chromatin integrity was monitored by acrylamide gel electrophoresis of histones as well as by its ability to serve as a template for *in vitro* DNA dependent RNA synthesis¹¹. Oviduct cytosol (10 mg protein ml.⁻¹, 105,000g supernatant fraction) was prepared, prelabelled with ³H-progesterone as described previously^{3,4}, and incubated with 50 µg chromatin for 1 h at 4° C. Oviduct chromatin (●), reconstituted oviduct chromatin (○), intact erythrocyte chromatin (▲), reconstituted hybrid chromatin containing the DNA from erythrocyte chromatin and the histones and acidic protein from erythrocyte chromatin (■) were used. See text for methods of chromatin reconstitution.

thymus. Finally, oviduct chromatin from which the histone had been removed still displayed a more extensive binding than spleen chromatin stripped of histone. We therefore felt that histones themselves were not primarily responsible for the specificity of receptor binding.

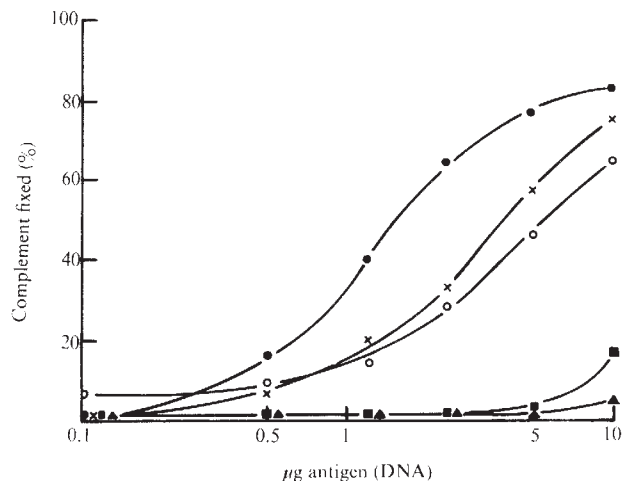


Fig. 2 Complement fixation by the intact and reconstituted chromatin from oviducts of immature chicks treated for 15 days with DES or from erythrocytes of 2 yr old hens. The antigens were intact oviduct chromatin (●), reconstituted oviduct chromatin (○), intact erythrocyte chromatin (▲), reconstituted hybrid chromatin containing the DNA from erythrocyte chromatin and the histones and acidic protein from oviduct chromatin (x), and reconstituted hybrid chromatin containing the DNA from oviduct chromatin and the histone and acidic protein from erythrocyte chromatin (■). The antisera were made using, as the antigen, the DNA-acidic protein complex (DNA-AP) prepared from oviduct chromatin from chicks treated for 15 days with DES as described elsewhere¹². An equal volume of complete Freund's adjuvant was added to the DNA-AP, the mixture homogenized and injected weekly for 6 weeks into rabbits (male New Zealand). The rabbits were bled by cardiac puncture 7 days after the last injection, and the resulting serum (diluted 1 : 800) was used for determination of micro-complement fixation by the method of Wasserman and Levine¹⁴. The anticomplementarity (binding of the complement in the absence of antiserum) was tested in the whole range of concentrations used and subtracted from the total complement fixed. The dissociation-reconstitution was performed as described in the text.

Preliminary experiments in which the dissociated chromatin was treated with RNAase before reconstitution suggested that chromosomal RNA was also not of great importance for the extensive binding. If during reconstitution the acidic (non-histone) proteins of chromatin were removed, however, the chromatin lost most of its capacity to bind the progesterone-receptor complex⁸.

Acidic Proteins of Chromatin recognize Steroid Receptors

We next performed more detailed experiments to test the importance of the acidic proteins in regulating receptor binding. We dissociated the acidic proteins (and histones) from the chromatin of oviduct and erythrocytes by a single treatment using 2.0 M NaCl-5.0 M urea (pH 8.3) by a modification of the conditions described by Bekhor *et al.*¹⁰. Approximately 90% of the acidic proteins were dissociated from the DNA by this procedure. It was possible to reconstitute the histones and most of the acidic proteins (AP) back to the DNA by gradient dialysis in the presence of divalent cations (0.001 M Mg^{2+}) and sodium bisulphite.

The acidic proteins and histones of the chromatin of one tissue were thus reconstituted with purified DNA from another tissue

to form hybrid chromatin (Fig. 1). Oviduct cytosol, containing the 3H -progesterone-receptor complex, was then incubated separately with the intact, reconstituted, or hybrid chromatin. Intact oviduct chromatin again bound progesterone-receptor while very little binding was noted to erythrocyte chromatin (Fig. 1). Reconstituted oviduct chromatin bound in a manner quantitatively similar to that of intact native oviduct chromatin. But when the AP of erythrocyte was inserted onto the oviduct DNA during reconstitution, this hybrid lost its ability to bind receptor and resembled native erythrocyte chromatin. Conversely, insertion of AP from oviduct into erythrocyte chromatin bestowed binding capacity to this hybrid chromatin resembling that of native oviduct. These experiments demonstrate that the inherent acceptor capacity of target tissue chromatin for the hormone receptor of that tissue can be transferred to a non-target DNA through transfer of the AP fraction. The data are consistent with the fact that the acceptor specificity of oviduct chromatin is due to the presence of specific sites on certain acidic proteins. We cannot rule out the possibility, however, that certain specific acidic proteins could determine the conformation of DNA in chromatin necessary for accepting the hormone-receptor complex.

Immunochemical Identification of Chromatin Acidic Proteins

To monitor the acidic protein fraction during reconstitution and to substantiate chemically our ability to form genuine

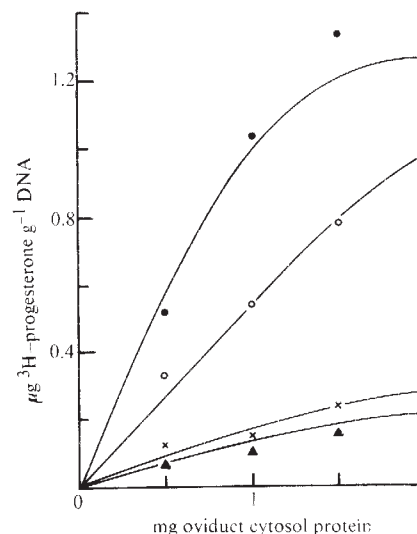


Fig. 3 Binding of 3H -progesterone preincubated with oviduct cytosol to the reconstituted oviduct chromatin devoid of selected acidic protein fractions. The histones and acidic protein fraction AP₁ were removed by the high salt and urea at low pH. After centrifugation, the DNA-acidic protein pellet was dissolved in dilute buffer, and the insoluble acidic protein fraction AP₂ removed by low speed centrifugation. The soluble DNA with the remaining acidic proteins was then treated with high salt-urea at pH 8.5 to dissociate the acidic protein fraction AP₃. Ultracentrifugation sediments the DNA bound to fraction AP₄. This last fraction (AP₄) can only be removed from the DNA with phenol or protease treatment. In this procedure, the relative distributions of total acidic protein into the various subfractions are as follows: AP₁, 15%; AP₂, 40%; AP₃, 35%; AP₄, 10%. The binding of 3H -progesterone preincubated with oviduct cytosol to the reconstituted oviduct chromatin devoid of selected acidic protein fractions was then assessed. Approximately 50 µg (expressed as DNA) of reconstituted oviduct chromatin containing all acidic proteins (●), or minus AP₂ (○), minus AP₂ and AP₃ (x), or minus AP₂, AP₃, and AP₄ (▲) were each incubated with increasing amounts of labelled cytosol. These reconstituted chromatin were prepared by reconstituting the histone and AP₁ back to DNA preparations containing either (1) AP₂, AP₃ and AP₄; (2) AP₃ and AP₄; or (3) no protein. These preparations were obtained using methods described in Fig. 2.

hybrid chromatin, a quantitative and qualitative assay for native chromatin-AP complex was necessary. An immunochemical method using quantitative microcomplement fixation was developed^{12,13}. Antibodies were produced in rabbits against dehistonized chromatin, that is, acidic protein-DNA complexes (AP-DNA) isolated from chromatin of chick oviduct nuclei. In these conditions, the antibody recognition of the dehistonized chromatin from different preparations of chick nuclei is highly specific and reproducible. Neither pure DNA nor histones cross-react in this assay. The complement fixation is a measure of antibody reaction with AP-DNA (antigen) complex (Fig. 2).

Reconstituted oviduct chromatin containing AP from oviduct differed slightly from native oviduct in its ability to react with antibody, apparently because the reconstitution of AP and DNA is not complete (Fig. 2). The ability of the antibody specifically to recognize AP from oviduct is supported by the lack of appreciable reaction with AP-DNA from native erythrocyte chromatin. Most important, however, is the fact that marked complement fixation is acquired by erythrocyte DNA when it is complexed with oviduct AP as a hybrid chromatin. Conversely, a hybrid chromatin of oviduct DNA and erythrocyte AP loses its antigenicity. Thus, by this immunological criterion the AP fractions of oviduct and erythrocyte chromatin can indeed be switched during reconstitution to form true hybrid chromatin (Fig. 2) and lends further credibility to the data shown in Fig. 1.

Fraction AP₃ contains the "Acceptor Sites"

It was then of interest to attempt fractionation of the total AP so that acceptor sites might be localized to a specific fraction.

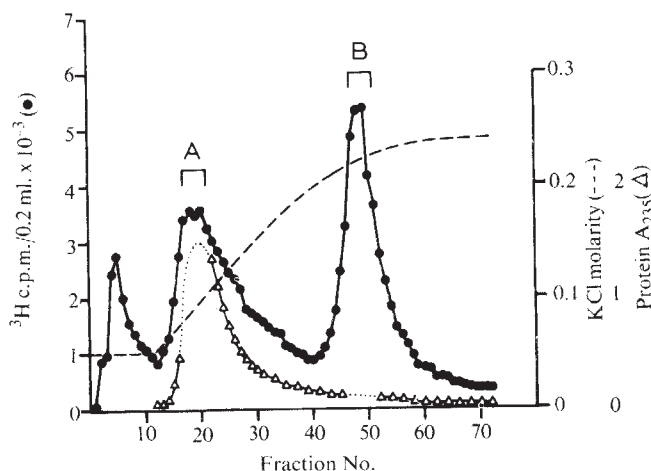


Fig. 4 Chromatography of oviduct progesterone receptor components on DEAE-cellulose. Oviduct cytosol receptor preparation in TESH (0.01 M Tris-HCl, 0.001 M EDTA, 0.012 M thioglycerol, pH 7.4) was labelled with 10^{-7} M ^3H -progesterone for 1 h at 0°C . The receptors were precipitated in 30% saturation buffered ammonium sulphate, collected by centrifugation, and redissolved in TESH. This material was applied to a 3 cm diethylaminoethyl (DEAE)-cellulose column washed with TESH containing 0.05 M KCl. After the material not adsorbed to the resin eluted (which contained no hormone binding activity and co-chromatographed with free progesterone), a KCl gradient (---) in TESH was used to elute the column. Two peaks of radioactivity (●), A and B eluted; peak A coincided with the peak of protein (Δ).

When binding of the progesterone-receptor complex to oviduct chromatin was monitored during sequential extraction of these various AP fractions, the results shown in Fig. 3 were observed. A small reduction in chromatin binding capacity was noted after removal of AP₂ but the principal loss of binding ability occurred after removal of AP₃. The small loss in hormone binding capacity with removal of AP₂ probably occurred because a small amount of AP₃ was also removed

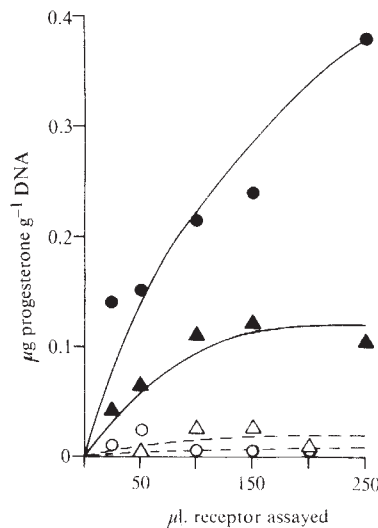


Fig. 5 Binding of purified progesterone receptor components to chromatin. Increasing amounts of labelled receptor components A and B prepared by DEAE-cellulose chromatography (Fig. 4) were incubated for 1 h at 4°C with 50 μg (expressed as DNA) of chromatin. The figure shows binding of component B to oviduct (●) and spleen (Δ) chromatin and of component A to oviduct (○) and spleen ($\triangle$) chromatin in the hormone binding assay described in the text.

during extraction of AP₂. No further reduction occurred after removal of AP₄. We therefore conclude that AP₃ contains the great majority of acceptor molecules for the receptor complex. Future emphasis will be directed toward purification of these potentially important proteins of the target-tissue genome.

Receptor contains Subunits

We next examined the chemical organization of the receptor molecule itself. During purification, the progesterone receptor was subjected to ultracentrifugation, ammonium sulphate fractionation and gel filtration. When the partially purified receptor was passed through a DEAE-cellulose ion-exchange column, the receptor appeared to dissociate into two forms eluting at 0.10 and 0.22 M KCl respectively (Fig. 4). The two peaks (A and B) were present in each oviduct tissue preparation in equimolar amounts. The two peaks were not interconvertible and maintained their separate identity on rechromatography. The components were found to have identical hormone binding kinetics ($K_d = 10^{-10}$ M) and steroid specificities, suggesting that the same class of hormone binding site is involved on both A and B, although they differ in certain stability properties¹³. It seemed that A and B could represent subunits of the native receptor complex. Both A and B components were found following *in vitro* or *in vivo* labelling and were noted also to be present in oviduct nuclei following administration of progesterone. We then questioned whether one of these receptor components might selectively interact with the acidic protein acceptor molecules in the nuclear chromatin of target tissues.

Chromatin Binding Residues in the B Component

We quantified the binding ability of the purified receptor components A and B by incubating them as described above with oviduct and spleen chromatin. When increasing amounts of A and B were added to chromatin, fraction B demonstrated enhanced binding to oviduct chromatin compared with a non-target tissue such as spleen (Fig. 5), similar to the behaviour of crude cytosol receptor. In the same conditions fraction A, although stable in the assay conditions, showed no binding to any of the chromatin preparations. It is thus possible that fraction B, 3,000-fold purified at this point, may be the

"specifier" subunit of native receptor responsible for the specific binding to the target cell chromatin acceptor sites.

If the hormone-receptor complex is actually the inducer unit for steroid hormone modulation of nuclear RNA transcription, then this initial binding to the genome may prove to be important in understanding the biochemical mechanisms of steroid hormone action.

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Tectonic Significance of the Afar (or Danakil) Depression

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The Afar depression results from the separation of the Arabian and Nubian plates, with generation of new oceanic crust. Volcano-tectonic spreading axes form part of broad uplifted structures. The Gulf of Aden and Red Sea rifts constitute one and the same tectonic megastructure; but the distinct characteristics of the East African rift system suggest that it does not pertain to the megastructure.

THE Afar depression is the junction area of three most important tectonic structures: the Red Sea, Gulf of Aden, and East African rift systems (Fig. 1). Recent developments in plate tectonics have drawn attention to this region, the significance of which has been interpreted differently by different authors. Controversy exists about stratigraphy, tectonic trends, tectonic structures, presence or absence of oceanic crust, amount of spreading and so on.

The Afar depression being the only emerged portion of the Red Sea-Gulf of Aden structure and a key region for their interpretation, it is rather surprising that no systematic geological research had been undertaken seriously before 1967. Extensive geological field work has been carried out since that date by our CNRS-CNR research team. The results obtained thus far allow a sound reconstruction and permit an elimination of some of the interpretations formerly proposed.

Evidence of Crustal Separation

The amount of spreading has been interpreted either as corresponding to the total separation between the Red Sea

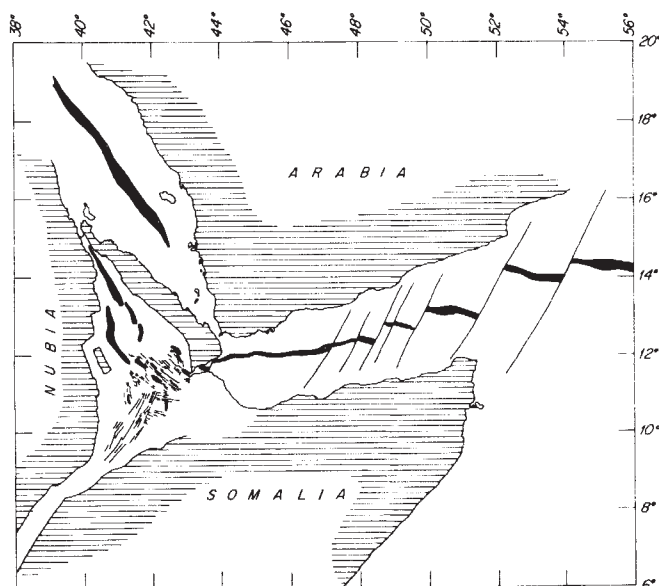


Fig. 1 Location of Afar depression in relation to the Red Sea, Gulf of Aden and East African rift systems. Zones of generation of new oceanic crust are shown in full black. Main faults of the Lake Abbe area are recorded to show the relations between the Afar depression and main Ethiopian rift.

and the Gulf of Aden coastlines^{1,2} or as restricted to the width of the median trench^{3,4}. The nature of the spreading mechanism has also been questioned⁵.

The main tectonic feature of northern and central Afar (north of 11° N, Fig. 2) is a prevalingly NNW-SSE set of distensive faults⁶, with typical rift-in-rift structures disposed in an *en échelon* pattern^{7,8}. Volcanic rocks of oceanic type characterize the rift-in-rift axes (Fig. 2).

These axial units are built up over NNW-SSE trending fissures. The petrological evolution, from mildly alkalic basalts to peralkaline rhyolites, with numerous intermediate members (such as ferrobasalts and dark trachytes), is clearly related to the volcanological evolution. Differentiation is more developed where more evolved volcanic structures are observed, that is, Erta'Ale⁹ or Tat'Ali ranges; in other ranges, such as Alayta¹⁰, N. Loggio or Asal, evolution only reached the dark trachyte member and volcanological characteristics are also of a lower evolutive state. Volcanic rocks of these ranges can be explained in terms of crystal fractionation in low and decreasing fO₂ conditions¹¹. Isotopic data confirm the oceanic nature of these rocks, which clearly differ from those located in the volcanic units situated closer to the rift's margins, where crustal influence is observed¹⁰. All data obtained so far suggest the absence of sialic crust within these axes, which are made up of newly created oceanic rocks.

These Afar axial zones of crustal separation appear at approximately the same latitude as the dying out of the main

trough of the Red Sea, close to latitude 15° (Fig. 1); it may thus be concluded that Afar is but an *en échelon* disposed structure pertaining to the main Red Sea system^{7,8,10}. This displaced segment of the Red Sea tectonic axis extends over two degrees latitude from Alid to Lake Afrera (13°), over the Salt Plain and the Erta'Ale range. In this segment the amount of crustal separation increases from north-southward, while a corresponding reduction is observed within the Red Sea south from latitude 15°.

South of Lake Afrera the axis of the depression is split into two *en échelon* volcano-tectonic units, both with a Red Sea trend: Alayta and Tat'Ali ranges (Fig. 2). To the south, the geology becomes more complicated: the axes of crustal separation appear less evident and seem to be fragmented into several different short rift-in-rift structures coexisting at the same latitude (11°-12°), as for N. Loggia, Dama Ale, Manda and Asal.

Detailed studies show that important uplifts are affecting Afar rift-in-rift axes. Actual uprising movements sometimes predominate even in collapsed structures. This is demonstrated by the horsts observed along the central axis of northern Afar (Alid-Erta'Ale)^{9,12} as well as by upper Pleistocene and Holocene submarine volcanoes of northern and central Afar, which are now exundated and have even reached altitudes several hundred metres above sea level (Mount Asmara)^{13,14}; in French Territory, Marinelli (personal communication) has pointed out an obviously arched structure in the Asal graben¹⁵;

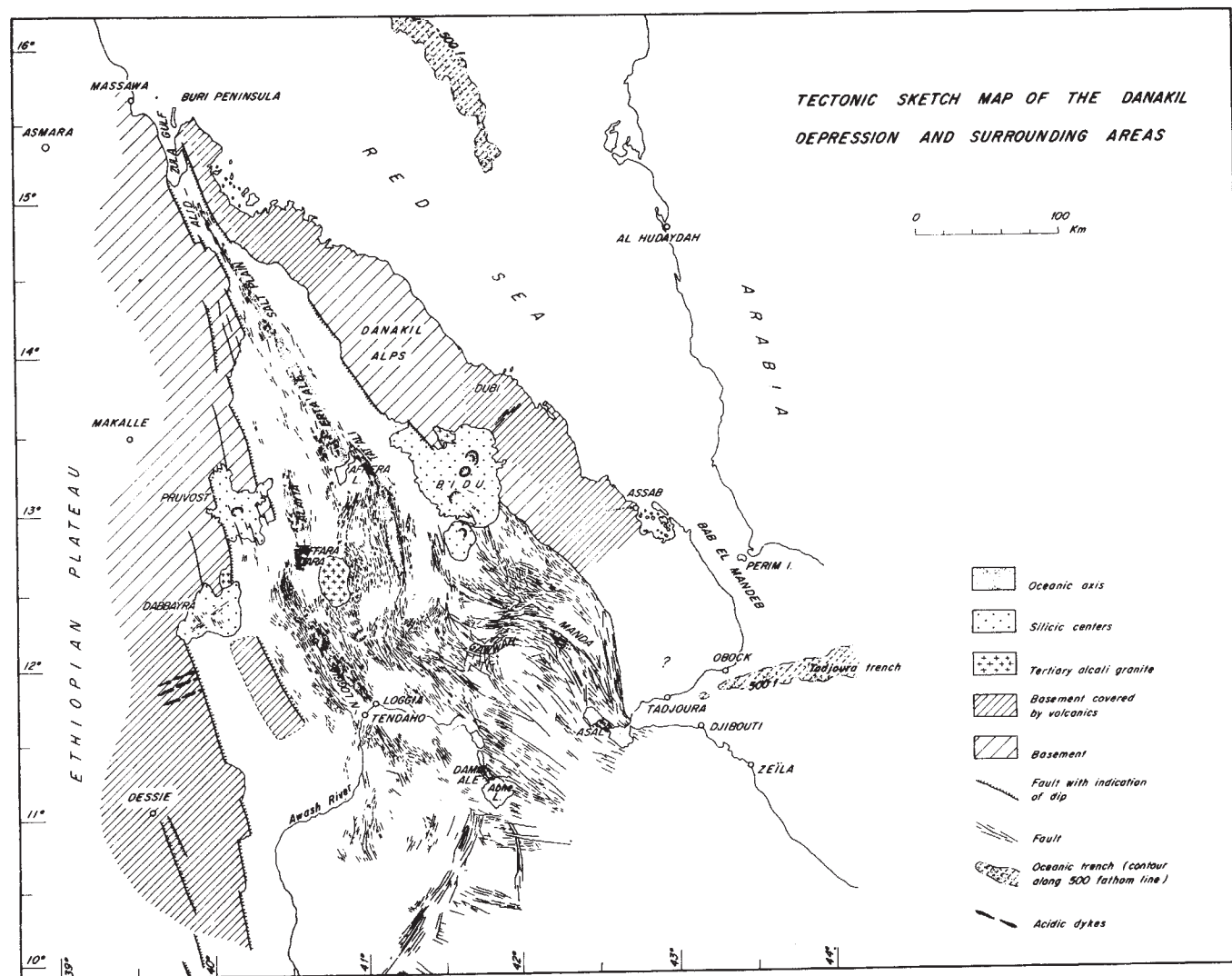


Fig. 2 Tectonic sketch map of Afar.

and the axial trench of the latter is spectacularly uplifted, at a rate that has been calculated on ^{14}C evidence to be of about 90 m during the past $5,800 \pm 150 \text{ yr}^{12}$.

These apparently down-faulted grabens, where uprisings are actually observed, are marked by normal distensional faults and gaping fissures which allow subcrustal magma to well up. It may accordingly be considered that this arching up is related with both oceanic volcanism and observed separation of tectonic blocks.

Red Sea–Gulf of Aden Megastructure

Magnetic and gravity surveys in the western part of the Gulf of Aden show that the main negative central magnetic anomaly does not pass from the Gulf of Aden into the Red Sea across the straits of Bab-el-Mandeb, but through the Gulf of Tadjoura and the Afar depression. Moreover, recent seismological profiles conducted in the Gulf of Tadjoura and the Asal area have shown the presence of abnormal upper mantle, probably of mid-oceanic ridge nature, similar to that found in the Gulf of Aden¹⁶. These geophysical data confirm that the main axis of regional expansion actually passes through the Afar depression.

East from longitude 47° E , the Gulf of Aden tectonic trends, as demonstrated by hypsometric and magnetic surveys^{17,18}, are chiefly ESE–WNW or SE–NW. The Somali highlands bordering the Gulf of Aden to the south exhibit a largely prevailing SE–NW oriented fault system¹⁹. West from the E–W oriented trench of Tadjoura, these “Sheba” trends resume in central Afar: the magnetic anomalies veer from E–W in southern Afar, to SE–NW in central Afar, and eventually to the SSE–NNW in northern Afar²⁰. Tectonic field evidence suggests either that the Sheba trend criss-crosses the NNW Red Sea trend (in the Kyu-Enkebba region or the area north from the Ghoubbet-al-Kharab), or that it progressively bends northward until it merges imperceptibly with the Red Sea direction; this one itself deviates to a NW–SE direction in central Afar¹². These facts strongly suggest that the Gulf of Aden and the Red Sea rift systems constitute one single megastructure, the inflexion area lying within the Afar depression.

Sheba–Red Sea Megastructure Junction with East African Rift

The NNE–SSW tectonic trend of the African rift system is only observed in the south-western corner of the Afar depression. The SSW–NNE tensional faults of the East African rift system disappear almost immediately once they have crossed the first lineament of the Red Sea–Gulf of Aden megastructure; they are not present in Eastern Afar. Geological evidence is supported by geophysical data which show that neither magnetic nor gravity anomalies exist following NNE trending (that is, African rift trend) in either central or northern Afar. The “Wonji fault belt”, allegedly continuing throughout Afar up to the Red Sea²², disappears north-west of Lake Abhe in our opinion. Consequently, we feel that there is no need to consider further the Afar depression as a “funnelling out” of the East African rift system^{1,21,23,24}.

Rotation of Danakil Horst

The separation of the Danakil horst from the Nubian block was accompanied by a rotation of the Danakil horst. This can be deduced from a series of arguments such as a study of the faulting directions of the scarps, a comparison of the Danakil block and the Ethiopian plateau geology, the progressive increase of the volcanicity from north southwards in northern and central Afar, the splitting of the single oceanic axial graben into two parallel ones south from 13° N , the presence of crustal rocks splinters in between the oceanic rift-in-rift structures, and palaeomagnetic data²¹. Geological evidence points to a rotation angle of $18^\circ \pm 10^\circ$ with a pole located close to Alid volcano (Fig. 3).

Patterns of the scarps on either side of the depression (Fig. 3) differ: while the western border of the Danakil horst is comparatively straight with a NW–SE direction, the Ethiopian scarp is not simply a N–S oriented tectonic feature, as suggested by many published maps, but actually consists of a series of *en échelon* normal faults with the characteristic NNW–SSE Red Sea direction. This is the actual trend of the depression's western limits, and consequently reduces by a matter of 12° to 15° the angle of the apparent relative rotation of the Danakil horst, as it might be erroneously deduced from maps showing an alleged N–S direction for the Ethiopian scarp^{23,24}.

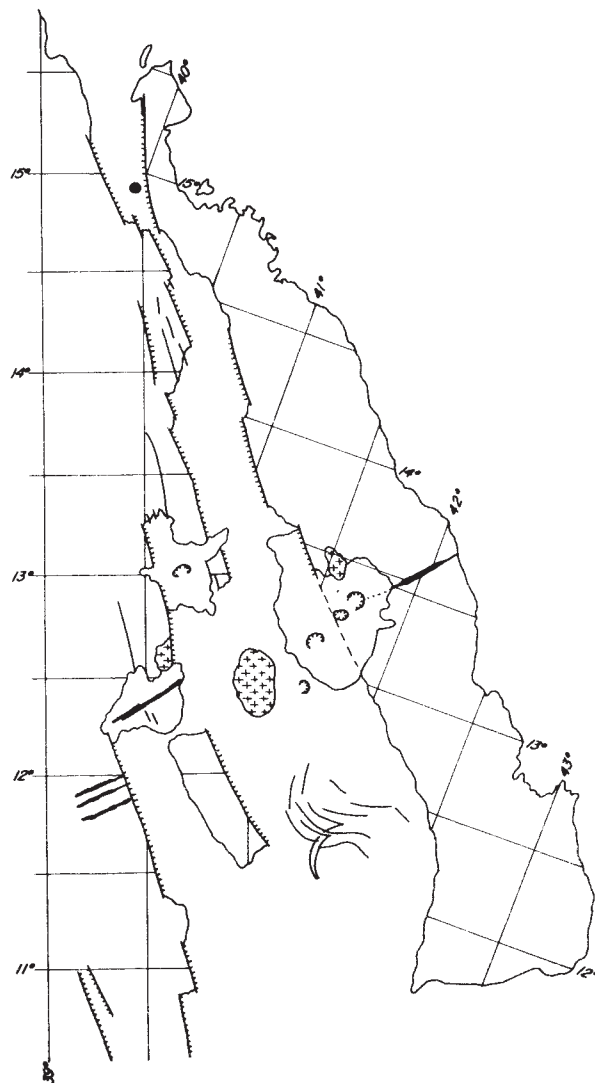


Fig. 3 Position of Danakil block after a clockwise rotation of 18° , with pole located at Alid volcano (full point).

These tectonic features account for continental crust splinters located between the *en échelon* disposed oceanic axes. The Danakil block can be considered to be such a splinter, and the largest one, because it is located between the main trough of the Red Sea and the longest Afar *échelon*; smaller splinters of Ethiopian plateau have been mapped closer to the Ethiopian scarp, and the Miocene Affara-Dara alkali granitic body and the surrounding rocks may be considered the exposed portion of a somewhat larger sliver²⁵.

The presence of still hidden intrusive magmatic bodies, on the other hand, may be responsible for tectonic discrepancies, such as the curvilinear grabens of central Afar. These features

have been considered as rotational structures²⁶, but this hypothesis is in apparent contradiction with field observations, which show only tensional fissures, to the exclusion of any shear-stress features.

Trans-rift Lineaments

Transform or transcurrent faulting could be expected in an area of oceanic nature, and some authors²² have described such trans-rift lineaments in the Afar depression. Unfortunately, our observations are in formal contradiction with this, as none of these mapped transcurrent faults has been actually observed in the field nor detected on aerial photographs. A few volcano-tectonic features, subperpendicular to the Red Sea trend, are present in both the Ethiopian scarp (Dabbayra) and the Danakil horst (Dubbi)²⁵. Being definitely tensional, they cannot be mistaken for shear faults. Moreover, they do not affect the depression proper, and possibly correspond to pre-rift crustal tectonics; they fit perfectly, both in orientation and position, a reconstitution assuming an angle of rotation of about 18 degrees for the Danakil horst (Fig. 3). These tensional fault patterns have been rejuvenated by recent tectonics. The variation of the direction of faulting of the Danakil block north and south of Bidu-Dubbi transverse weak zone may be interpreted assuming a different angle of rotation north and south from this line, the rotation angle of the southern part being a little wider (20°) than the northern one (18°). Therefore, contrary to widespread opinions^{22,23}, one cannot consider this Bidu-Dubbi structure as the continuation of the East African rift system (Wonji fault belt) further.

Conclusions

The Gulf of Aden and Red Sea rift systems do make up one single megastructure, the tectonics, magmatism and significance of which are different from those of the East African rift.

Crustal separation zones (oceanic rifts) of the Red Sea and Gulf of Aden are connected by the axial volcano-tectonic rift-in-rift structure of the Afar depression (Fig. 1).

The Danakil continental horst is supposed to have moved away from the Nubian plate with a dextral rotation of 18° ±

10°. The exact amount of spreading cannot be deduced from geological basis only, and supplementary geophysical data are required to solve the problem quantitatively and should be required to any sound interpretation of the Afro-Arabian area in terms of plate tectonic mechanisms.

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Iranian Geology and Continental Drift in the Middle East

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This article summarizes Iranian geology and continental drift in the Middle East. The relation of the two suggests that plate tectonics satisfactorily explain the geological development of the Middle East.

the models for the Alps or the Himalayas. Some of these difficulties were discussed by Stöcklin¹ who concluded that Iran had a peculiar type of Alpine tectonics. On the other hand, the position of Iran in the continental reconstructions has not been very clear. It has been assumed usually that Iran belonged to Eurasia or was situated between the two continents. The exact boundary between the two and their relation with the mountain ranges of Alborz and Zagros have not been explicit, however. I have already suggested² that Iranian geology should be analysed in the context of continental drift, and in this article I shall emphasize some of the aspects which can be explained by consideration of continental drift and plate tectonics.

GEOGRAPHICALLY the Iranian mountains belong to the Alpine-Himalayan chain but their structures do not clearly fit into

Geologically, Iran can be divided into two regions, the Zagros folded belt and the rest of the country (Fig. 1). In the Zagros folded belt³ quiet and conformable sedimentation continued from Cambrian to Pliocene times. Only gentle epeirogenic movements and salt diapirism affected this belt before the Late Tertiary Zagros orogeny when the sediments became folded into a series of parallel anticlines and synclines. The fold axes trend in a north-west-south-east direction and the amplitudes of the folds decrease towards the south-west away from the border of the Zagros and the rest of Iran⁴.

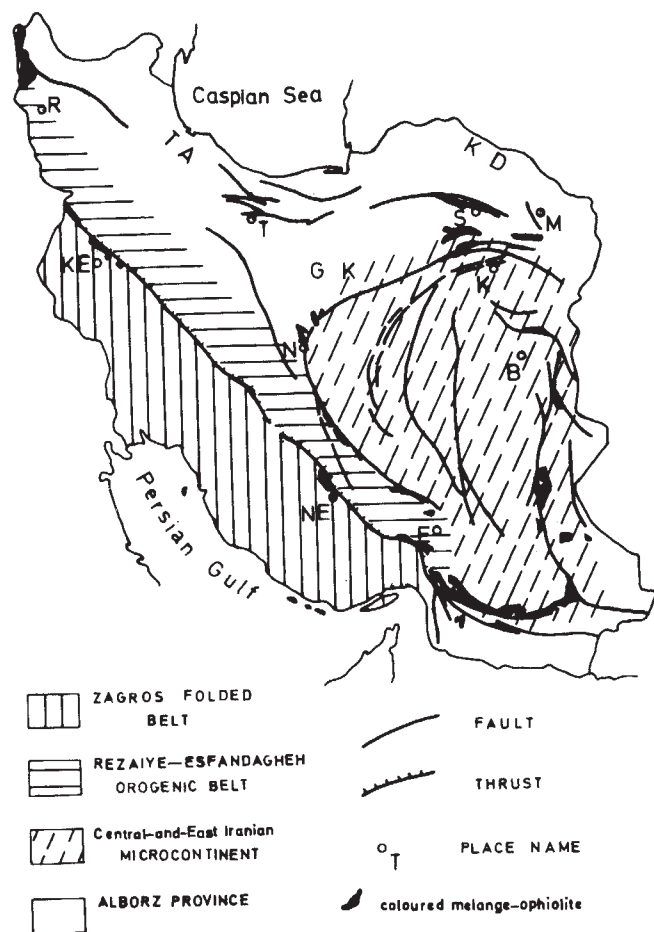


Fig. 1 Schematic divisions of Iranian geology. B, Birjand; E, Esfandagheh; GK, the Great Kavir; K, Kashmar; KD, the Kopet Dag mountains; KE, Kermanshah; M, Mashhad; N, Nain; NE, Neyriz; R, Rezaie; S, Sabzevar; T, Tehran; TA, Talesh area.

The geological history and tectonic development of the rest of Iran have been completely different from those of the Zagros. The epeirogenic movements were more severe and there have been orogenic events, intrusions and metamorphism. There are angular unconformities, important disconformities and sedimentary gaps, rapid facies changes both laterally and vertically, and widespread volcanicity in many parts and at different times^{1,5}. The rest of Iran has generally similar characteristics everywhere but can be divided into three provinces which are generally bordered by "coloured melange-ophiolites".

(1) The Rezaie-Esfandagheh orogenic belt, which runs parallel to the Zagros from north-west to south-east and joins the Taurus orogenic belt in Turkey⁶, was previously¹ called the "Sanandaj-Sirjan Ranges" but it is here given a more

precise geographical and geological nomenclature. This belt is separated from the Zagros by a narrow zone of deep thrusts and reverse faults (the Zagros crush zone). In some parts along this zone (particularly in Kermanshah and Neyriz areas⁷) certain unusual rock assemblages (coloured melange-ophiolites) are present. These assemblages were thrust out of the narrow crush zone onto the Zagros in Late Cretaceous times^{8,9}.

(2) Central-and-East Iran includes "Central Iran. S.Str." and "Lut Block" of Stöcklin¹. Its outline is approximately like an inverted triangle. The south-west limit of this geological province is marked by a major fault associated with coloured melange-ophiolites. The fault runs from Nain to north-east of Esfandagheh¹⁰⁻¹². In the north-west of this province, a fault runs from Nain to north Kashmar¹³ to south Mashhad area. Coloured melange is present in some parts along this fault. But other faults with similar trend exist further north-west. The limit of this geological province may thus be situated in the centre of the Great Kavir and is covered by younger formations. The eastern limit of the province is approximately along the north-south-running East Iranian ranges¹. Coloured melange is present along these ranges, but they also extend partly inside the province in Birjand area^{14,15}. On both the eastern and southern borders, flysch deposits are extensively developed which cover the older rocks. They are chiefly of Tertiary, but also of Cretaceous, age¹⁵.

(3) The Alborz includes the sinuous ranges of the Alborz mountains in North Iran and a parallel zone to the south of the mountains. The mountains extend into the Little Caucasus and north-eastern Turkey and also to the Hindu Kush and the Karakorum. A very important geological feature of this province is the presence of "depressions" (for example, the southern Caspian Sea) where thick sediments overlie a thick "basalt" layer¹⁶⁻¹⁸. The southern border of the Alborz is not clearly defined by coloured melange-ophiolite rocks. Such rocks of Late Cretaceous age are present in the Sabzevar region. Ophiolites of pre-Jurassic age may exist in Talesh area (R. G. Davies, personal communication). In extreme partly north-eastern Iran the Kopet-Dagh mountains have had a geological history similar to that of the Zagros folded belt. There is, however, no distinct border separating the Kopet Dag from the Alborz as is the case between the Zagros folded belt and the rest of Iran.

Continental Drift in the Middle East

For the present discussion Iranian geology is closely related to the past movements of Eurasia, Africa (including Arabia), India, and therefore to the geology and past history of the Indian Ocean. These movements include both the southern and northern continents and cannot be considered locally. It is now generally agreed¹⁹⁻²⁶ that the Tethyan Ocean, a few thousand kilometres wide, existed in the present area of the Middle East in the Mesozoic and probably in the Palaeozoic or even earlier. It separated the Afro-Arabian and Eurasian continents (Fig. 2). The former was part of Gondwanaland and was situated much further south, with India, Australia, Antarctica and South America. Afro-Arabia gradually moved northwards at least since the Permian, causing the contraction of the Tethyan Ocean. On the other hand, the movements of Eurasia with respect to the Tethyan Ocean were of a smaller magnitude. There was a slow westward movement in the Mesozoic and an eastward one in the Cainozoic. It also underwent a gentle northward drift in the Cretaceous.

The exact original position of India and its later movements have been interpreted in different ways. It was probably situated to the east of Africa. Some old oceans might have existed between East Africa and India^{22,23} or between India and Australia^{19-21,25} or in both regions²⁶. The pole positions for the later movements of India are not definite. This

is apparent when comparing the proposals of Veevers *et al.*²³ with the other reconstructions. Also its northward drift might have been preceded by a southward movement²⁰.

Geological Consequences

The general northward drift of Afro-Arabia continued in the Mesozoic and resulted in the narrowing of the Tethyan Ocean. During this time a subduction zone had developed in the Tethys where the oceanic crust was sliding down into the mantle. The subduction zone (Tethyan Trench) was elongated in a west-north-west to east-south-east direction. It seems that the subduction zone was partly along the southern edge of the Eurasian continent (resulting in the development of a trench-cordilleran belt) and partly away from the continent (resulting in a trench-island arc)²⁷. The northward drift of Afro-Arabia continued until all the oceanic crust had disappeared and the edge of the Afro-Arabian continent reached the subduction zone. This event took place in the Late Cretaceous before the Maestrichtian^{8,9}. At this time ophiolites were emplaced on the edge of the Arabian continent (the Zagros crush zone and the Oman Peninsula). The cordilleran belt is now seen as the Rezaie-Esfandagheh belt. Its continuation as an island area is seen in Oman^{28,29} and probably as "continental fragments"²⁶ in Oman Sea and alongside the ophiolite belt in north-western Himalayas. The ophiolites in the Zagros crush zone are not therefore related to the Zagros orogeny and to the opening of the Red Sea as has been proposed⁴.

The ophiolites of the crush zone are more widespread in the Kermanshah and Neyriz sectors. In those two localities granites of batholithic dimensions are present in the Raziye-Esfandagheh orogenic belt. The thrusting of the ophiolites on top of the Zagros sediments⁷ was therefore facilitated by isostatic uplift at the time when subduction was coming to an end.

The Zagros sedimentary basin (the present folded belt) was the shelf of the old Afro-Arabian continent. It was stable geologically and was the site of quiet (mainly carbonate) sedimentation. Detrital sediments derived from the north-east were deposited in the Zagros sedimentary basin from Early Tertiary (Amiran and Kashkan formations)³. The sources of these sediments were the ophiolites and the orogenic belt which had joined the Afro-Arabian continent in the Late Cretaceous.

In the northern continents, there were only short intervals of drift in the Mesozoic. These movements resulted in the partial break-up of Eurasia. Small microcontinents (for example, Central-and-East Iran) were produced, surrounded by narrow Red-Sea type ocean basins (Fig. 3). The remnants of these small oceans are seen as the coloured melange-ophiolites around the Central-and-East Iranian microcontinent and in the Sabzevar and Rezaie areas. These oceans were therefore younger than the Tethys, which had probably existed in the Palaeozoic or even in the Precambrian. Triassic and Jurassic fossils are present in the coloured melange along the Zagros^{8,9} (the remnants of the Tethys) but have not been reported from the Nain area¹⁰.

It seems that the northward drift of Afro-Arabia continued in the Early Tertiary and resulted in the closing of the young small ocean basins. The coloured melange in the Nain area includes some Eocene rocks¹⁰. This explains why the ophiolite emplacement did not cause folding of the Zagros sediments in the Late Cretaceous. The rest of the compression was more easily taken up by the closing of the small oceans. Finally Afro-Arabia and Eurasia became connected by a mosaic of old microcontinents and island arcs. Only shelf seas still existed over parts of the region. The seas gradually regressed towards the south-east.

There was a pause in the compression in Eocene times when the seafloor spreading in the Indian Ocean was slow or had

stopped²⁶. This event coincides with the profusion of volcanicity over the Iranian region.

The extrusions were more developed along zones of weakness or of thinner crust where the tension (that is, relaxation of compression) enhanced the volcanicity. This explains the origin of the belt of Tertiary volcanic rocks which extend across the country from north-west to south-east.

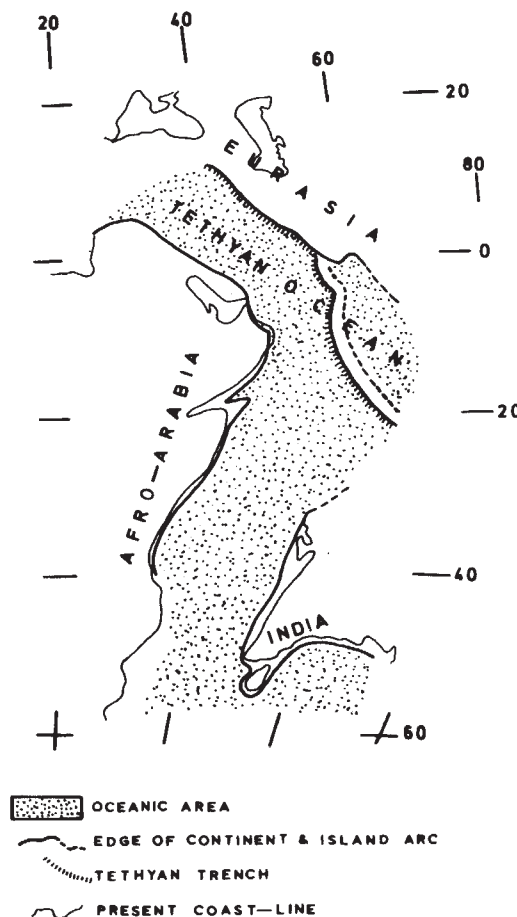


Fig. 2 Schematic arrangement of continents and oceans in the Middle East area at the end of the Triassic period. The general position of continents after Dietz and Holden²¹.

India reached the subduction zone in the old Tethys at the end of the Cretaceous as indicated by the ophiolites in the Himalayas³⁰. India's northward drift continued in the Tertiary, however. This resulted in underthrusting of its continental crust below that of Eurasia and increased crustal thickness in Tibet and Eastern Hindu Kush Ranges³¹. The differential movement between India and Arabia was taken up along the Owen Fracture Zone in the Indian Ocean³², the Quetta line in West Pakistan^{30,33}, and the Chaman fault Zone in Afghanistan (ref. 31 and P. Bordet, personal communication). There was therefore severe compression and complication of the geology due to the continental underthrusting in the Eastern Hindu Kush and the Himalayas. In the Iranian region the compression during the Tertiary was a great deal less. The geological structures of the Late Mesozoic and Early Cainozoic are still visible in this region.

It is obvious that the continued northward drift of India could have caused some additional compressions, deformations,

and rotations of the mosaic of plates in Eastern Iran (for example, Central-and-East Iranian microcontinent) and in Afghanistan. India's movement caused some uplift in these areas. Their erosion products were transported towards the south-east of Iran where they are now seen as the widespread flysch deposits.

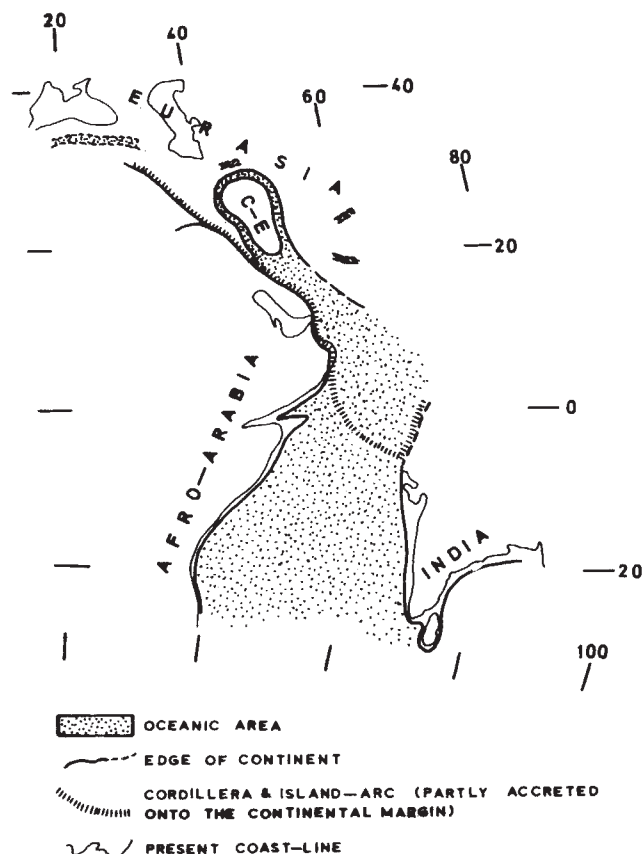


Fig. 3 Schematic arrangement of continents and oceans in the Middle East area before the end of the Cretaceous period. C-E, Central-and-East Iranian microcontinent. The general position of continents after Dietz and Holden²¹.

Another period of compression, in the Late Tertiary (Zagros orogeny), involved compression in a north-easterly direction as well as north-west-south-east dextral movements⁷. It was related to the opening of the Red Sea and Gulf of Aden^{4,34-37} and to the general south-eastward movement of Eurasia²¹. These movements are continuing today. The strongest forces were felt at the boundary of the Arabian continent. The Zagros thrust was reactivated and the rest of Iran was partly underthrust¹ by Arabia. The sediments of the Zagros were pushed from the northeast and were folded into anticlines and synclines. The compression also affected the other parts of Iran, causing folding, reactivation of old faults and the present seismicity³⁸. The Late Tertiary drift involves the separation of an East African-Somali Basin plate from a plate containing the Arabian Peninsula, the Oman Sea and possibly India³⁷. The total spreading on both sides of the Owen Fracture Zone has been almost equal but some differential movement has taken place as indicated by the bottom topography and distribution of earthquake epicentres³² and by their focal mechanism solutions^{38,39}.

It may be further argued that the "depressions" in the Alborz and the southern Caspian Sea are a consequence of

the small drift of Eurasia. The effect of processes in the mantle (for example, local convection, concentration of radioactive isotopes, development of local hot spots, differentiation and phase changes) would be more pronounced on the stationary or slowly moving plate. The continental crust underwent "basification or oceanization"⁴⁰ by intrusion of high-density dyke swarms and underwent subsidence¹⁸. Such phenomena were much less effective on the fast moving plate of Afro-Arabia.

A detailed discussion of this work⁴¹ was presented at the meetings of the Geological Survey and the Oil Companies in Tehran. I thank Dr Stöcklin and other colleagues of the Geological Survey of Iran and Drs Cann and Matthews (England) and Dr Le Pichon (France) for many discussions and comments and for their help.

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LETTERS TO NATURE

PHYSICAL SCIENCES

New X-ray Pulsar near the Crab Nebula

ON October 17, 1970, a new X-ray pulsar was observed with a balloon-borne X-ray telescope launched from Palestine, Texas. The detector consisted of three proportional counters filled with xenon at 2.5 atm. The total effective area was 480 cm², and the sensitivity extended from 30–100 keV with an average efficiency of 80%. A multigrid collimator provided a field of view of 3° FWHM in elevation and 12° FWHM in azimuth. Data were recorded from the Crab Nebula between 4.00 and 8.00 CST. Each count was telemetered to the balloon base and recorded on analogue magnetic tape with a resolution in pulse arrival time of 0.1 ms. The magnetic tape also contained reference timing signals, stable to better than 5 parts in 10⁹. The Crab pulsar NP 0532 was clearly detected¹.

Because the field of view included the known radio pulsar, NP 0527, which has a period^{2,3} of 3.74549 s, we carried out a search for this period. The data were initially analysed using the Cooley–Tukey fast Fourier technique⁴. In order to perform this analysis, a portion of the data was selected and divided into six parts, each containing 512 one-second averages. When the power spectrum for each part was plotted, each of these six parts showed a peak at 134/512 Hz, which corresponds to a period of 3.821 ± 0.015 s. No other peak was seen on more than two plots. In each record the peak at channel 134 was approximately three times higher than the average. There is an *a priori* probability that in 12 out of 256 channels there will be a peak that high; this is confirmed by the distribution of peaks in each graph. The random probability that six independent groups of data will each show a peak in the same channel is 256 (12/256)⁶, that is, less than 3 parts in 10⁶. The result of adding the six power spectra together is shown in Fig. 1A. The amplitude distribution of this composite power spectrum is very close to a normal distribution (not logarithmic as we expect for a single power spectrum). An analysis of this distribution shows that the peak in channel 134 is 5 standard deviations above the mean; the random

probability of this occurrence is one part in 10⁶. The results of a similar analysis of data, acquired before and after the Crab passed through the field of view, are shown in Figs. 1B and C. No indication of a period of 3.821 s was present during these times.

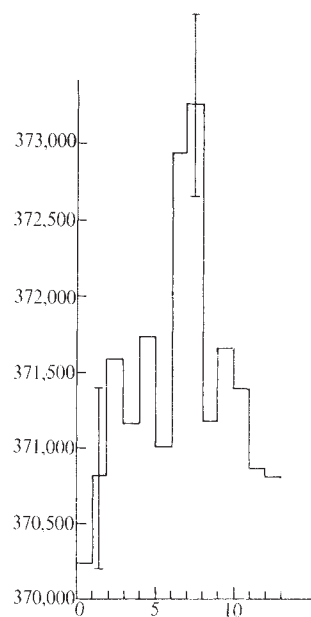
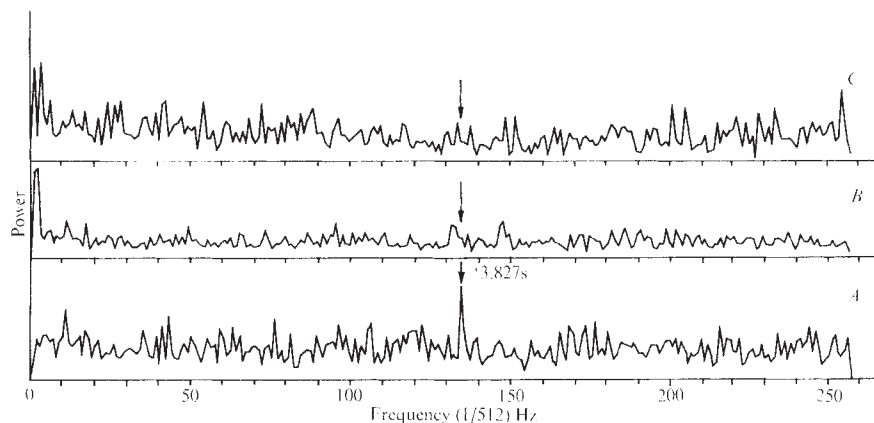


Fig. 2 The phase histogram for the X-ray period of 3.8266 s. Each channel covers 299 ms. There are 4,000 counts under the peak, accumulated during 5,100 s of flight.

Although the resolution of the power spectrum analysis is only 30 ms, we found a more exact period by plotting the respective pulse profiles for different periods. For the period 3.8266 s, each of the tapes obtained when the Crab was in the field of view showed an approximately 2.5 σ peak. Using IRIG-B time recorded on each tape, the data were combined. The resultant phase histogram is shown in Fig. 2, where counts

Fig. 1 Power spectra for three different observing times: A, when the X-ray detectors have the Crab Nebula in the field of view (3° × 12° field of view); B, before the Crab entered the field of view (20° off); C, after the Crab had passed through the field of view (60° off). The arrow shows where the 3.827 s peak should have appeared. It appears only on curve A (5 σ peak). Each power spectrum, A, B and C, is constructed from 3,072 s of counts.



per channel are plotted against the channel number (each channel represents 299 ms). The average signal of the two highest channels is 4.2 standard deviations above the mean of all thirteen channels. The probability for this to occur by chance is less than 1 part in 30,000. The total pulsed emission was 4,000 counts (1.6×10^{-3} counts $\text{cm}^{-2} \text{s}^{-1}$) for the 5,100 s of accumulation. This is 0.27 times the intensity measured from NP 0532 (ref. 1).

This frequency might be a beat frequency between the 30.212 Hz of NP 0532 and nearby frequencies ($30.212 \pm (3.8266)^{-1}$ Hz). Alternatively, the second harmonic of NP 0532, at 60.424 Hz, could beat against $60.424 \pm (3.8266)^{-1}$ Hz. We searched our data for such nearby frequencies, but none was found. Furthermore, a beat frequency occurring between the second harmonic of the Crab pulsar and the 60 Hz present in the ac line would give a period of 2.41 s and not 3.82 s. Measurement of the frequency of the ac line as recorded on the data tape indicates that its true frequency was 60.00 ± 0.01 Hz.

If we accept this new evidence of X-ray pulsation, it may be associated with the central star in the Crab Nebula, NP 0532, with the other known radio pulsar near the Crab, NP 0527, or it may originate in a third pulsar in the vicinity of the Crab. The observed X-ray period of 3.8266 s is 2% greater than the radio period of NP 0527. Possibly differential rotation near the velocity of light circle could produce such a difference.

We have searched older NP 0532 rocket data (1–10 keV) for evidence of pulsation, without success. But the length of observing time encompassed only a few cycles of the pulsar. Rappaport *et al.*⁵ reported only an upper limit for NP 0527 (1.5–6 keV) from a rocket flight. Giacconi has informed us (private communication) that no evidence of pulsation from NP 0527 is present in UHURU satellite data (2–7 keV) covering several 100 s passes.

If further observations confirm that NP 0527 is an X-ray pulsar, our present models of X-ray pulsars must be revised; a difference in period between the radio and X-ray pulsations would be even more surprising. It is common to most theoretical models of pulsars as spinning neutron stars that X-rays would be expected only from the youngest and fastest pulsars. But NP 0527 is the slowest known radio pulsar. Furthermore, in the case of NP 0532, the X-ray, optical and radio periods are identical to the limits of the precision of measurement. Only one other X-ray source, Cen X-3, is known to have a persistent pulsation⁶. But thus far it has not been detected at radio frequencies. Its period, ~ 5 s, is even longer than that of NP 0527. The suggestion has been made that Cen X-3 could be a white dwarf near the limit of rotational instability. If the new source is not associated with NP 0527, it may be an object like Cen X-3.

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Identification of GX3+1 from Lunar Occultations

THE position of GX3+1 has previously been reported by Schnopper *et al.*¹ with sufficient accuracy to allow the times of lunar occultations to be predicted to within about ± 3 min². This uncertainty in the predicted time can be covered by the relatively brief observation period of the flight of a sounding rocket. Two of the present cycle of lunar occultations were predicted for the rocket range at Woomera, Australia, and were successfully observed: the first on September 27, 1971, by the Leicester X-ray Astronomy Group, and the second on October 24, 1971, by the Mullard Space Science Laboratory, UCL.

At the time of the September occultation, the Sun was 83.3° from GX3+1. A sideways-looking proportional detector of 840 cm² area was mounted on a Sun-pointing Skylark rocket (SL 1002). This detector has a slit collimator of 2.5° FWHM (in the direction of the rocket axis) by 15° FWHM (in the roll plane). The detector, which was 5 cm deep, was fitted with a 12 μm melinex window, and filled with a 90/10 mixture of argon-methane gas at 1 atm. The detector electronics was sensitive in the energy range 1–18 keV. Pulse height information was transmitted in real time, each output being coded according to pulse rise time so that the particle background could be reduced during analysis.

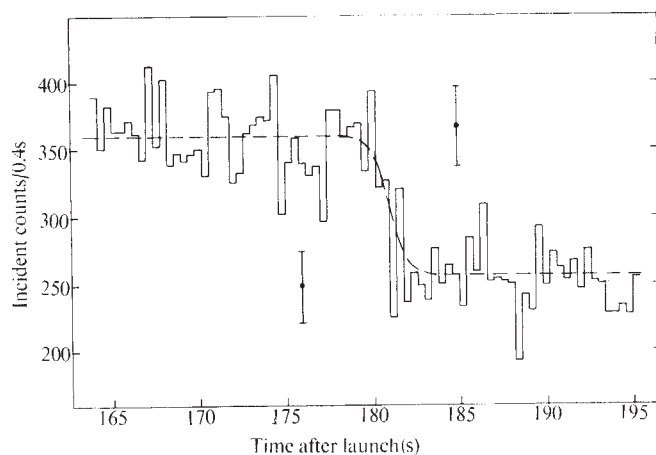


Fig. 1 SL 1002. The counting rate in the energy range 1.3 to 7 keV for the period 164 to 196 s after launch in 0.4 s channels. Error bars are 1σ . The dotted line represents the expected transition if the source were of extent 1.5 arc s FWHM.

At the time of the launch (11 h 30 m local time) the solar vector was within 10° of the magnetic vector, so the magnetometers of the normal Skylark Sun-pointed ACU could not be used to control the roll. But the strong X-ray source Sco X-1 was 62° from the Sun and 9.6° away in roll from GX3+1, conveniently placed to use a modification of the Sco-Sensor roll control system which has been used successfully in two previous Leicester rocket flights³.

The Sco-Sensor on the September 27 flight consisted of two proportional detectors, each 110 cm² in area and collimated to 7° FWHM (in roll) by 18° FWHM, with the centres of the fields of view 7° apart in roll. The detector electronics produced a pulse train in which positive logic pulses corresponded to an X-ray incident on one of the detectors, within the energy range 1.3 to 10 keV, while negative logic pulses corresponded to similar events in the other detector. The roll control unit, which was activated after the Sun had been acquired, initially caused the rocket to execute a controlled roll of 15°s^{-1} . When Sco X-1 was observed the roll was halted and the rocket was stabilized on this source such that the two halves of the Sco-Sensor received equal counting rates. The roll limit

cycle, as measured during the flight, was 10 arc min (peak to peak).

During the disappearance of the source the combined effect of the motions of the rocket and of the Moon resulted in the Moon having an apparent initial motion of 1 arcs s^{-1} , gradually slowing down until it appeared stationary at the end of the 300 s observation period. During this time the Moon's limb apparently moved through 140 arc s. The launch time was calculated to centre the occulted area of sky on the previously known mean position.

The rocket was launched at 02 h 00 m 25 s UT and was fully stabilized on the Sun and Sco X-1 by 96 s after launch. The counting rate was then steady until 181 s after launch when a drop in incident rate of $260 \text{ counts s}^{-1}$ was observed. The rate then remained steady until 400 s after launch, when roll control was lost because of atmospheric absorption of the Sco X-1 flux. Energy calibration of the main detector was made at the beginning and end of flight, using a ^{55}Fe X-ray source. The background counting rate was above that predicted, partly because other sources were not completely excluded from the field of view and partly because of atmospheric fluorescence as a result of the low elevation of the Moon.

Analysis of the change in counting rate has given the time of the occultation as 02 h 03 m 26.0 $\pm$ 0.5 s UT, with 95% certainty. This corresponds to a positional error of $\pm 0.4 \text{ arc s}$. Fig. 1 shows the incident rate, corrected for dead-time effects in the energy range 1.3 to 7 keV, plotted with channels 0.4 s wide. The error bars are 1σ . Assuming that the source is diffuse and has a Gaussian intensity distribution, there is less than 5% probability that the source is larger in angular size than 1.5 arc s FWHM. The expected counting rate for this case is also shown in Fig. 1. Analysis so far gives the strength of the source as $0.45 \pm 0.05 \text{ photons cm}^{-2} \text{ s}^{-1}$ in the energy range 3–10 keV; the data are being analysed further for the energy spectrum, for time variation of the source, and the possibility of a low energy halo.

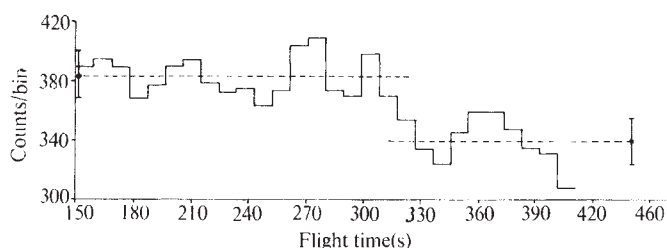


Fig. 2 SL 974. The counting rate from the 9.6° bin in azimuth, centred on the direction of GX3+1, summed over 9.42 s.

The second occultation was predicted to occur on October 24, disappearance at 11 h 09 m UT and reappearance at 12 h 10 m UT. By this time it was known that the disappearance on September 27 had been observed with SL 1002. It was decided to attempt to observe the disappearance on the later occasion also because the phase angles were such that this would yield a better position measurement. The disappearance occurred about two hours after sunset, the Moon being about one quarter full. Under these circumstances, only a spin-stabilized rocket could be used (SL 974), scanning the source direction once in each rotation. The effective exposure to the source was thereby reduced by a factor of ~ 60 , with a consequent increase in the uncertainty of the occultation time.

The payload was constructed so that either the appearance or the disappearance could be observed if necessary, although the elevation above the horizon of the source was different at these times. The elevation at disappearance was 31° , the source being at 54° from the roll axis. The centre of the field of view was set at 60° to this axis, the view field—which was defined by an egg-crate collimator—being 20° FWHM parallel

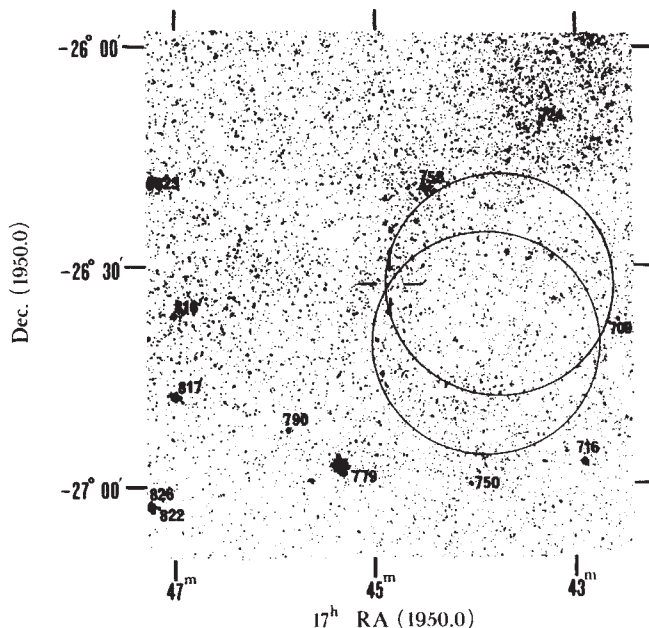


Fig. 3 The star field and the outlines of the Moon at the instants of occultation intersecting in the error circle of Schnopper *et al.*¹ indicated by four straight lines. The lower position of the Moon was that for the Leicester observation. The star numbers when prefixed by 185 are the SAO catalogue numbers; seven of these stars served as reference points for determining the RA and declination of the stars near to the point of intersection.

and 6° FWHM perpendicular to the spin axis. The detector and associated electronics were broadly similar to those used on SL 1002.

Data were analysed with a special data processor. Counts in the 3–10 keV band were grouped into bins of 3.2° in azimuth and 3.14 s in time, using as an azimuth reference zero crossings of a rocket magnetometer oriented perpendicular to the spin axis. A first analysis showed that a group of three adjacent azimuth bins gave evidence of a significant intensity change over the flight, while a neighbouring group of four bins did not. The counts in these two groups were therefore totalled, the latter group being processed (as a control group) as well as the former. The control group did not yield significant effects in the subsequent analysis, showing that no spurious effects were introduced in the treatment of the measurements. The measured direction of the centre of the three bins from that of GX3+1 was 10° , which is within the accuracy of the measurements.

A small correction (roughly $\pm 10\%$ or $\pm 1\sigma$) was made for the effect of the coning of the rocket. This was done by a superposed epoch analysis, using a coning period of 34.5 s determined from the magnetometers. Fig. 2 shows the counting rate as a function of flight time, the data being summed over three successive time bins. A 3σ change in count rate occurred at a flight time of about 317 s. Two different χ^2 tests were devised which allowed a time resolution of one time bin to be obtained and gave a more satisfactory basis for assessing the timing error. The three estimates of the occultation time lay within a range of 3 s. The estimated uncertainty in the time (corresponding approximately to a 2σ value) was ± 10 s.

Table 1 shows the best estimates of the times and standard deviations for the occultations and the positions of the rockets at those times.

These times and coordinates have been used to compute the positions of the limb of the Moon using the lunar ephemeris $j=2$ (ref. 4) with $\Delta T=40.5$ s. Fig. 3 shows the outlines of the Moon on the star field as seen by the rockets, with the leading edges intersecting in the error circle (radius

Table 1 Circumstances of Occultations

Experiment	Date and time (UT)				Longitude (east)	Latitude (south)	Altitude (km)
Leicester	Sept. 27, 1971	02 h 03 m 26.0 s	± 0.2 s		135° 06' 43"	30° 30' 34"	204.7
MSSL	Oct. 24, 1971	11 h 10 m 37 s	± 5 s		135° 52' 05"	30° 10' 01"	226.7

1.7') of the previous best determination of the X-ray position. Fig. 4 shows an enlargement of the region with stars 14 (about sixteenth magnitude) and 15 (about seventeenth magnitude) close to the point of intersection. The star numbers are those assigned in the paper of Kunkel *et al.*⁵.

Table 2 Standard Deviations of the Limb Positions

Experiment	Timing and rocket position	Lunar ephemeris	Combined error
Leicester	0.2"	0.3"	0.4"
MSSL	3.4"	0.3"	3.4"

Table 2 sets out the standard deviations in fixing the position of the limb of the Moon from the two experiments arising from errors in timing and the rocket position, and in the lunar ephemeris.

The positions of the limb were adjusted for the detailed profile using Watts's charts⁶: the standard deviation of 0.3" for the lunar ephemeris in Table 2 includes a part for the uncertainties in Watts's data.

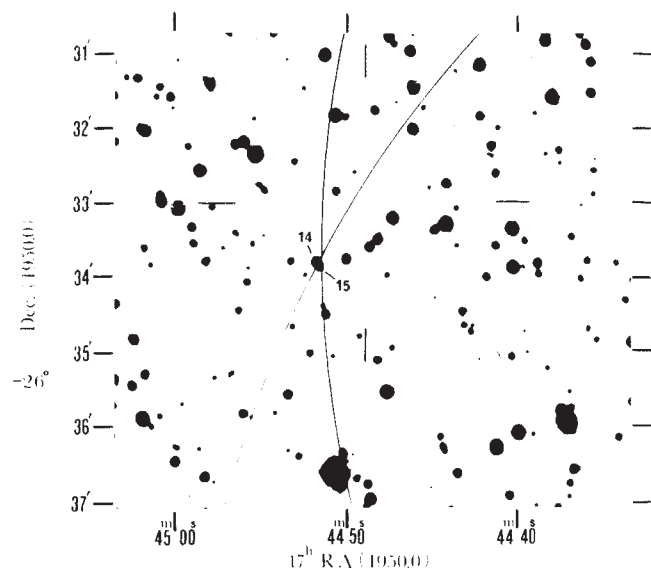


Fig. 4 An enlargement of the centre of Fig. 3 showing the intersection of the lunar limbs near stars 14 and 15. Copyright National Geographic Society—Palomar Sky Survey.

The positions of the faint stars in this area were not known accurately enough to be combined with the high resolution result from the occultations, so a photographic plate was taken at the Royal Observatory, Cape Town. The positions of images around the point of intersection of the two error boxes

were measured at the Royal Greenwich Observatory, taking seven of the SAO catalogue stars numbered in Fig. 3 as reference stars. The RA and declination of these objects were then derived using a plate reduction computer program developed by the Astrometry Department of the Royal Greenwich Observatory and used previously for determining accurate positions of radio sources. The formal standard deviation of the derived positions is 0.3"; however, it is more realistic to consider that the standard deviation of the measures is 0.5".

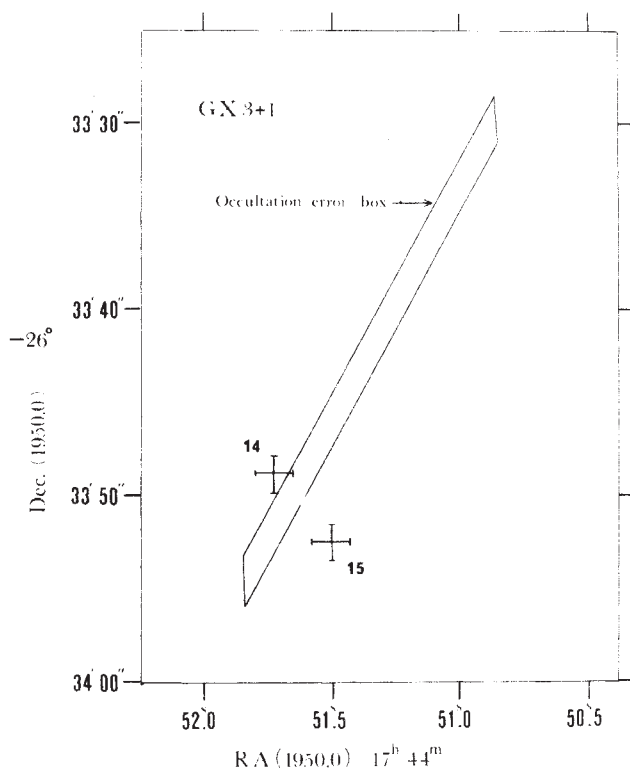


Fig. 5 The positions of stars 14 and 15 and the occultation error box resulting from the 2σ error in lunar limb position of $\pm 0.8''$ from the Leicester experiment and the $\pm 6.8''$ error obtained by MSSL.

Fig. 5 shows the measured positions of stars 14 and 15, the only objects visible near the two standard deviations error box resulting from the occultations; the error bars for the stars are also two standard deviations. Although the images of 14 and 15 are merged in Fig. 4, which is an enlargement of the Palomar Sky Survey print, they were clearly separable on the Cape plate which was used to measure their positions. Fig. 4 shows that there are no objects brighter than about twenty-first magnitude, other than 14 and 15, near enough the error box of Fig. 5 to warrant serious consideration as candidates. No object brighter than eighteenth magnitude was seen between stars 14 and 15 on the Cape plate. We conclude either that GX3+1 is associated with star 14, or that it is optically fainter than eighteenth magnitude and lying between 14 and 15, or it

is elsewhere in the error box of Fig. 5 and fainter than twenty-first magnitude.

We thank the staff of BAC and BAC(A) who prepared and launched the vehicles, and MSDS who developed the ACU and roll control unit for SL 1002. Technical assistance was provided by Mr R. Daldorph (Leicester) and Mr J. Holmes and Mr M. Savage (MSSL). We thank Mr G. A. Harding and Mr. G. M. Harvey of the Royal Observatory at the Cape for the photographic plate of the region, and Mr C. Guy and Mr C. A. Murray of the Royal Greenwich Observatory for measuring the plate and providing the plate reduction program. This work was supported by grants and a research studentship (for M. J. R.) from the Science Research Council. Both rockets were launched as part of the Science Research Council's national rocket programme.

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Plate Tectonics in the Caribbean

Freeland and Dietz¹ have presented a speculative geological history of the Caribbean using plate tectonic theory, and simultaneously Dickinson² suggested that two types of plate convergence may be distinguished by examining sedimentary and tectonic evidence. Geological data from the Caribbean support much of the proposed geological history but differ in significant details. On the other hand, the Caribbean seems to fit rather well the Dickinson distinction between plate convergence types.

Freeland and Dietz suggest that the pre-Jurassic Middle America areas of Yucatan and Nicaragua were nestled within the Gulf of Mexico, and that the Caribbean did not exist until Triassic times. During the Triassic the area fragmented into plates which rotated and moved into their present positions. Little can be criticized in the pre-Jurassic history presented, as no dated pre-Jurassic rocks have been mapped in the West Indies. Rocks as old as Middle Jurassic are exposed in Cuba³, but the oldest dated sedimentary rocks are Albian elsewhere in the West Indies. Metamorphic rocks and ultramafic rocks are pre-Albian in the Greater Antilles⁴ except in Cuba where they may be pre-Jurassic⁵. Ocean floor sampling in the Caribbean and Gulf of Mexico has revealed no rocks older than Jurassic and Cretaceous⁶. This perhaps suggests that no basins existed before that time, but it is equally possible that older oceanic rocks underlie the sampled horizons. Dengo⁷ and others^{8,9} have presented an interpretation of the geological history of the Yucatan-Guatemala and Nicaragua blocks which is internally consistent and which suggests that the two blocks were on opposite sides of a converging plate junction

during pre-Mesozoic times. It would be quite a coincidence if these two plates split apart, moved several hundred miles, and re-united in the same relative positions. Moreover, to move the blocks from the Gulf of Mexico into their present positions requires a peculiar spiral spreading motion¹ with north-western Yucatan pivoting on its north-west corner impelled by a spreading centre in the form of a circular arc stretching along the base of the continental shelf from Florida to southern Mexico near Vera Cruz. Perhaps a more successful history would follow Dengo and treat Middle America as a tectonic unit formed in the Palaeozoic as a convergent plate junction, and moved into its present location as a unit but not necessarily from the present Gulf of Mexico. The southern edge of the Palaeozoic North American continent was not necessarily at the present edge of the continental shelf in the Gulf of Mexico; it may have been further north next to the Appalachian orogenic belt. Palaeomagnetic data from Middle America should be a prerequisite for further speculation about large rotations of the one or more plates that can be defined.

The post-Triassic history of the Caribbean as suggested by Freeland and Dietz is more plausible. It is possible that the Cuban area began as a shelf between Yucatan and Nicaragua and moved northwards from a spreading axis along the northern Nicaraguan coast¹. Jurassic rocks in Cuba thicken and become more quartz-rich to the south³ indicating a southward source area. The Isle of Pines and the Trinidad Mountains, exposures of pre-Jurassic metamorphic rocks near to and in southern Cuba, could provide such a source without invoking the Middle American plates, however. Alternatively the Cuban metamorphics could be fragments of the Nicaragua plate moved to the north.

A more serious objection is the differing characters of the Yucatan basin and the Nicaragua rise. Freeland and Dietz suggest that these had the same origin: proto-Cuba and proto-Hispaniola split off from Middle America and moved north-eastward across the Caribbean, making volcanoes and islands as they went. But the Yucatan basin is oceanic with the closest resemblance to oceanic crust of any part of the Caribbean, while the Nicaragua rise is a shallow bank with a thicker crust and the moderate sized island of Jamaica. Why are they different? Moreover, the Greater Antilles are suggested to achieve their present position by small conveniently located spreading centres between each island pair: at least four separate spreading centres. This ignores the Cretaceous and early Tertiary tectonic pattern in the Greater Antilles which seems to show the results of a uniform force applied over a large area forming a complex but almost continuous ridge whose upper portions form islands, not a series of fault troughs and horsts oriented normal to the ridge as required by the Ptolemaic modifications to the plate tectonic hypothesis under discussion.

Dickinson's idea of activation and collision types of plate convergence at continental margins² offers a partial solution to the differing development of the Yucatan-Cuba and the Nicaragua-Hispaniola areas. The activation type of junction has oceanic lithosphere underthrusting the adjacent continental lithosphere, the normal type of plate convergence at a continental margin. This idea can be applied to intra-oceanic junctions if one lithospheric plate is less dense than the other; the less dense plate forms the overriding unit and a volcanic island arc is formed. The eastern Greater Antilles is an example, with a volcanic arc forming from Albian to Middle Eocene times.

The collision type of junction occurs when continental lithosphere is on the underthrusting side of a converging plate junction. It is less dense, and its buoyancy prevents it from descending into the Benioff Zone to any great distance. The convergence either stops, or in exceptional cases continues as continental lithosphere underthrusting the other plate as in the Himalayas, or there is a "readjustment of plate boundaries" (ref. 2). The third possibility can be applied to the tectonic history of the Caribbean.

In a general way, the tectonic history of the Caribbean can be viewed as the interaction of two lithosphere plates which changes its character progressively from west to east, from a converging junction to a lateral junction. Convergence began in the Antilles in Cretaceous, perhaps Albian time, with a less dense Caribbean plate overriding a more dense Atlantic plate along the Cuba-Virgin Islands trend. Perhaps the western end of the overriding plate moved north-eastward along the eastern edge of the Yucatan Peninsula¹. This movement and its volcanism continued until Turonian or Campanian time, when the Bahamas area of continental lithosphere entered the Benioff Zone north of Cuba and choked it, effectively welding the Cuban end of the Caribbean plate to the North America-Atlantic Plate. This is the collision type of convergence of Dickinson². The eastern part of the northward moving Caribbean plate continued to move, however, and a lateral junction began to develop in the Cayman Trench area, perhaps forming the Nicaragua rise and the Early Tertiary volcanism in easternmost Cuba, Jamaica, and the eastern Greater Antilles. Then, in mid-Eocene times, convergence and volcanism ceased in the Jamaica-Virgin Islands segment, but lateral movement continued. At roughly that time convergence and volcanism began in the Lesser Antilles. Thus the orogeny shifted eastward in the Caribbean, being progressively replaced by lateral motion at the plate boundary. This analysis could perhaps be expanded to Middle America, where convergence may have changed to lateral movement in the Jurassic¹. It is possible that the submarine Beata and Aves Ridges represent early zones of convergence related to the western Caribbean convergences, but those ridges are as yet almost unknown.

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Plate Tectonics in the Caribbean: a Reply

MATTSON's critique is in accord with most of our views¹. Here we answer most of his points by mentioning details omitted from the original paper because of space limitations.

(1) The region was essentially a landmass, part of Pangaea, before the end of the Triassic (190 m.y. BP, revised time scale)². The only pre-Jurassic movement was initiation of tension causing the Triassic grabens revealed by drilling in the subsurface along the northern and western edges of the Gulf Coastal Plain. The initial split was along the edge of the basin, not at the present coastline. Palaeozoic metamorphic rocks on

both the Yucatan and Nicaragua blocks, where they are not affected by subsequent compression and increased metamorphism in the Guatemalan foldbelt, are low grade, with affinities to rocks of Mexico³ and the Ouachita belt in Texas and Arkansas⁴. The major deformational episodes were synchronous with those of the Appalachians³. Accepting the Bullard reconstruction⁵, the Yucatan-Nicaraguan block must have fit either in the Gulf of Mexico or the Pacific Ocean if one is to avoid extensive overlaps on the South American continent. A fit within the Gulf seems most plausible. No continental areas created during the Mesozoic or Cainozoic were included in our reconstruction of Pangaea. Some Palaeozoic continental crust may, however, have been present in the gap to the south-west of the Bahamas. If so, this material has now been incorporated in the metamorphic-volcanic melange of the oldest parts of the Greater Antilles and is not at present recognizable.

(2) The Yucatan-Nicaragua blocks rotated to their present positions during the early and middle Jurassic, forming the proto-Gulf (the northern half of which is now filled with prograded Mesozoic and Cainozoic sediments); the proto-Gulf of Honduras; and the Guatemalan foldbelt.

(3) The Caribbean did not form until South America drifted away from Africa about 135 m.y. BP. By our interpretation, it was a spreading centre as the North and South American plates moved in separate directions, North America rotating anticlockwise relative to South America (see Fig. 6 of ref. 1). While the Caribbean opened, the Lesser Antilles subduction zone became active as the Atlantic, to the east, underrode the new seafloor.

(4) The early motion of South America was nearly due west with respect to Africa, but towards the end of the Lower Cretaceous it became more northerly. The later motion initiated compression and subduction along the Venezuela-Colombia coast. Initially larger than it is today, the Caribbean underwent closure during the Upper Cretaceous and early Tertiary.

An alternative hypothesis for the origin of the Jamaica-Hispaniola-Puerto Rico blocks would be orogenic construction along a single line of compression along the entire northern margin of the Caribbean. As closure progressed, subduction with attendant volcanism and metamorphism might have built these islands in their present locations instead of drifting them away from Nicaragua. Cuba, however, would still be formed by a spreading centre in the Gulf of Honduras, because Cuba is north of the Puerto Rico-Cayman trench and therefore outside of the Caribbean plate. The Yucatan Basin would be the result of this movement. The marginal basins thus produced are similar to others in crustal structure⁶. Although they attain nearly oceanic depths, they are usually generated by spreading characterized by the absence of a central ridge. In the Caribbean, however, certain rises may be fossil spreading centres.

(5) The Caribbean continued to close slowly. The climax of movement was during the middle Eocene when there was major tectonism on the northern and southern margins. The islands of the Greater Antilles then attained their present proportions.

(6) From the Eocene to the Present, the plate comprising the Caribbean Sea and the Nicaraguan block has been essentially welded to the northern margin of South America. Field evidence indicates no movement between them⁷, although there is some seismic activity⁸. The major present movement between the North and South American plates is a sinistral shear along the Puerto Rico-Cayman trench⁸. The lack of apparent offset between the Yucatan and Nicaragua blocks indicates limited movement.

Mattson's comments concerning plate boundaries seem to confuse the issue. The nomenclature of the convergences is not as important as the results of the motions. The western, northern, and eastern boundaries of the Caribbean during the Upper Cretaceous-Early Tertiary closing stage were inward-

dipping Benioff zones. It does not seem to matter whether the overriding portion of the plate was continental or oceanic; the results are similar. Part of the Caribbean plate was underthrust beneath South America. In turn, the Caribbean consumed ocean floor south of the Bahamas, east of the Antilles, and west of Nicaragua, Costa Rica, and Panama. During the last half of the Cainozoic, overthrusting ceased along the northern margin and a vertical shear zone took its place. To the west the Mexican trench is still active, although partially blocked by the Cocos ridge. The Lesser Antilles zone is still active, although it has probably migrated eastward.

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Activity at the Soufrière Volcano, St Vincent, West Indies, in October-November 1971

THE Soufrière Volcano in the north of St Vincent last erupted in 1902–03 (refs. 1, 2). This eruption was extremely violent: an estimated 1.4 km³ of pyroclast flow and fall was emitted, devastating the northern third of the island and killing 1,565 people. After this eruption, a lake was re-established in the summit crater, and since 1910 the lake water seems to have remained constant in level to within a few metres and in temperature to within a few degrees. The presence of fumaroles on the lake bottom is postulated to account for the fact that the lake temperature, which was measured regularly from 1946–1952, remained at about 4° C above the expected from the ambient air temperature³. Apart from this and since the occurrence between November 1945 and February 1946 of a swarm of local earthquakes⁴, several tens of which were felt around the flanks of the volcano, the Soufrière remained dormant until October 1971.

Recent abnormal activity was first reported on October 31, 1971, when the pilot of a light aeroplane noticed steam rising from the lake surface. This was confirmed by three further inspections from the air and by visits on foot to the crater rim made by three separate parties between October 31 and November 2.

On November 3 we descended the inner wall of the crater to the lake edge, where the water temperature was measured to be at 81.5° C, while the lake surface was estimated, by reference to known landmarks, to be at least 16 m above its normal level. From this date onward, the lake was visited regularly and between November 3 and 15 fluctuations in water temperature (minimum 79° C, maximum 82° C) have been insignificant, although the water level has continued to rise at a diminishing rate (Table 1).

During all periods of close observation, the lake surface was calm, although warm vapour was rising from its whole surface.

The vapour smelt faintly sulphurous. The lake water was turbid and uniformly yellowish-khaki in colour instead of its former bluish-green shade. No explosions were heard and no earthquakes were felt in recent months either by visitors to the summit or by permanent inhabitants of the lower flanks of the volcano.

A seismograph with peak gain of 13,500 at a period of 0.24 s was operating continuously up to October 13 and from November 1 onward at a permanent site 19 km to the south of the volcano. (This instrument, unfortunately, was out of action during the second half of October.) A second, similar instrument was installed on November 2 at Richmond, at the south-western foot of the volcano 6 km distant from the summit. No local earthquakes have been detected by either of these instruments during the period up to November 15.

No estimate of the lake level or temperature is available for the interval from September 19, 1971, when it was reported by an experienced observer to be at normal temperature (about 22° C) and level, until November 3 when we made our first measurement. A visitor to the summit on October 10, 1971, noted a sulphurous smell but no other unusual phenomena, although he did not report this until November 1.

We interpret the present situation to be the result of a large heat source, presumably magma, which has risen close to, or emerged at, the lake floor. The heating of the lake water to about 60° C above normal in a period of less than six weeks may be the result of either steam emitted through the lake floor or fresh lava poured out onto the lake floor or a combination of both processes. Soundings made in 1958 (ref. 2) showed the lake to occupy an inverted, truncated cone with a volume of 75 million m³. The cubical expansion of water caused by heating from 22° C to 82° C is 2.6%, which corresponds to 2 million m³ or an increase in water level of about 2 m. It is necessary, therefore, to account for the remaining increase in water level of at least 14 m, equivalent to a volume increase of 12 million m³. Assuming that this increase results entirely from displacement by a lava flow of this volume, and that it reached the surface at a temperature of 1,200° C, we calculate that approximately one-third of the total available heat has already been transferred to the lake water. Alternatively, if all the heat was added to the lake in the form of steam at 150° C which condensed before reaching the lake surface, then we calculate that with complete efficiency of heat transfer, 8 million m³ of new water will have been added to the lake; or taking two-thirds efficiency of transfer to allow for heat losses at the lake surface and into the crater walls and floor, then the observed volume increase of 12 million m³ could be accounted for wholly by the addition of condensed steam. Thus either mechanism for heating the lake is plausible and it may be that some combination of the two has taken place.

If the case of heating primarily by a new lava flow on the lake floor is considered, then the shape of this body and its thickness (about 60 m) are known, as also is the amount of heat which it has yielded. Provided that the lake undergoes no future increase in level (that is, no further influx of lava or steam occurs), then the expected rate of cooling of the water may be estimated. A large body of hot lava introduced into the lake within the past 8 weeks has probably not yet lost more

Table 1 Changes in Crater Lake Level between November 3 and 15, 1971

Date	Level above November 3 datum (cm)	Rate of increase since previous measurement (cm/day)
November 3, 1971	0	
4	30	30
5	58	28
7	91	15.5
9	122	15.5
11	122	0
13	137	7.5
15	150	6.5

than half of its original heat. It will therefore continue to transmit heat to the water which will take longer to cool than if the heat was a steam supply which has now ceased.

If, on the other hand, the lake level continues to rise, then there will be no immediate basis for distinguishing whether lava or steam has been added, and it is unlikely that a definitive explanation of the recent phenomena will be given until the lake has cooled to the temperature at which a small boat can be launched in order to carry out a new sounding survey.

The apparent absence of local earthquakes is surprising because even if magma has not reached the lake floor it must have risen close to it, and both the 1812 and the 1902 eruptions were preceded for more than a year by earthquakes felt locally¹. We therefore conclude that during the present crisis only a relatively small volume of magma has so far risen through the upper crust, and that this magma probably had low viscosity and gas pressure which allowed it to migrate upward with the minimum of crustal deformation. Careful and regular monitoring of the volcano's activity is continuing and any new developments should be promptly detected.

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Heavy Metal Concentration in Coastal Waters

No data are available for the concentration or distribution of trace metals in coastal waters to the west of England and Wales, including the toxic metals such as cadmium, lead, copper and zinc. This deficiency is particularly serious considering the nature and amounts of natural runoff and waste effluent present in these waters.

The preliminary results presented here illustrate the general levels and distribution of some trace metals in Liverpool Bay, Cardigan Bay and the Bristol Channel, and are part of a survey made during the period 1969–1971 to study the seasonal chemical variation in these regions. The selected areas are characterized by regionally different effluents. The dominant sources of material controlling trace metal levels in Liverpool Bay are domestic and industrial effluents, whilst Cardigan Bay is relatively free from industrial effluent and, because of the low population density, little domestic waste is present. In this region, the runoff from the mineralized zones and sites of former mining activity is the main source of trace metals. In the Bristol Channel, industrial and domestic effluent carried by the River Severn and the River Avon together with the runoff from the mineralized zone in north Devon contribute to the trace metal occurrence and distribution.

Sampling grids were located for these regions. They were made up of twenty-seven stations in Liverpool Bay (extending to the Isle of Man), twenty stations in Cardigan Bay and forty-four stations in the Bristol Channel. Trace metal determinations were made on 10 l. samples (taken from a depth of 5 m) using a pulse polarographic method, after the concentration of trace metals by chelating resin¹.

The concentration levels and distribution of copper, lead, cadmium and zinc are shown in Table 1 and Fig. 1a–d. The levels of these metals decrease outwards in all the three regions to a level comparable with or approaching that of an average seawater². There is also a significant build-up of trace metals within these regions, particularly near the River Mersey and the River Ribble, in Tremadoc Bay and in the eastern part of the Bristol Channel. This build-up seems to be controlled by both effluent and the circulation of water in the regions.

No unusual concentration of cadmium is encountered in Liverpool Bay, but the copper, lead and zinc levels increase inshore. The copper and zinc distributions indicate that these are contributed by the Mersey effluent. The high patches of lead are attributed to the runoff from north Wales, the effluent from the Mersey and Ribble and from activities on the Isle of Man. A large area of the Bay is characterized by a high zinc concentration, five to ten times its concentration in the open seawater of the region.

Table 1 Average Levels and Range of Concentrations ($\mu\text{g/l.}$) of Copper, Lead, Cadmium and Zinc in Liverpool Bay, Cardigan Bay and the Bristol Channel Surface Waters

	Cu	Pb	Cd	Zn
Liverpool Bay				
Mean	1.45	1.74	0.27	11.86
Lowest	0.90	0.66	0.14	2.30
Highest	3.03	4.17	0.74	47.6
Cardigan Bay				
Mean	1.72	2.24	1.11	7.46
Lowest	0.98	1.12	0.48	3.63
Highest	4.02	3.53	2.41	19.65
Bristol Channel				
Mean	2.07	1.18	1.13	9.98
Lowest	1.02	0.35	0.28	3.57
Highest	4.74	5.06	4.20	21.42
Average S.W. *	3	0.03	0.11	10

* From ref. 2.

High levels of zinc, copper and cadmium are found chiefly in Tremadoc Bay—an area of very limited circulation. These metals are contributed by the runoff from the River Glaslyn which flows through the mineralized zone of Snowdonia. High patches of lead, cadmium and zinc are also found in the middle of the Bay and these are due to the unusual "vortex-like" circulation pattern in this part of Cardigan Bay controlling the runoff from the River Dovey.

A high level of lead is found between Swansea Bay and the north Devon coast. This may be caused by the runoff from the mineralized zone in north Devon. The effect of this runoff is also reflected in the high levels of copper and zinc around the north Devon coast. High levels of copper and zinc are also found in Swansea Bay and the eastern part of the Bristol Channel, possibly attributable to industrial and domestic waste disposal in these regions. The concentration of cadmium in the Channel is the highest encountered in the Irish Sea coastal waters, levels of over 4 $\mu\text{g/l.}$ (or thirty to forty times that of open sea levels) being found in the eastern parts of the Bristol Channel. The main source of cadmium is the Avon and Severn effluent which enters an area of very limited circulation in the channel. The cadmium is possibly derived entirely from industrial effluent, as there are no known natural cadmium deposits in the region. The distribution pattern shows that the water movement along the channel is responsible for the "streaming" of cadmium along the northern part of the channel corresponding to the freshwater movement.

The results presented here show that the distribution of trace metals in coastal waters is controlled by both the amounts

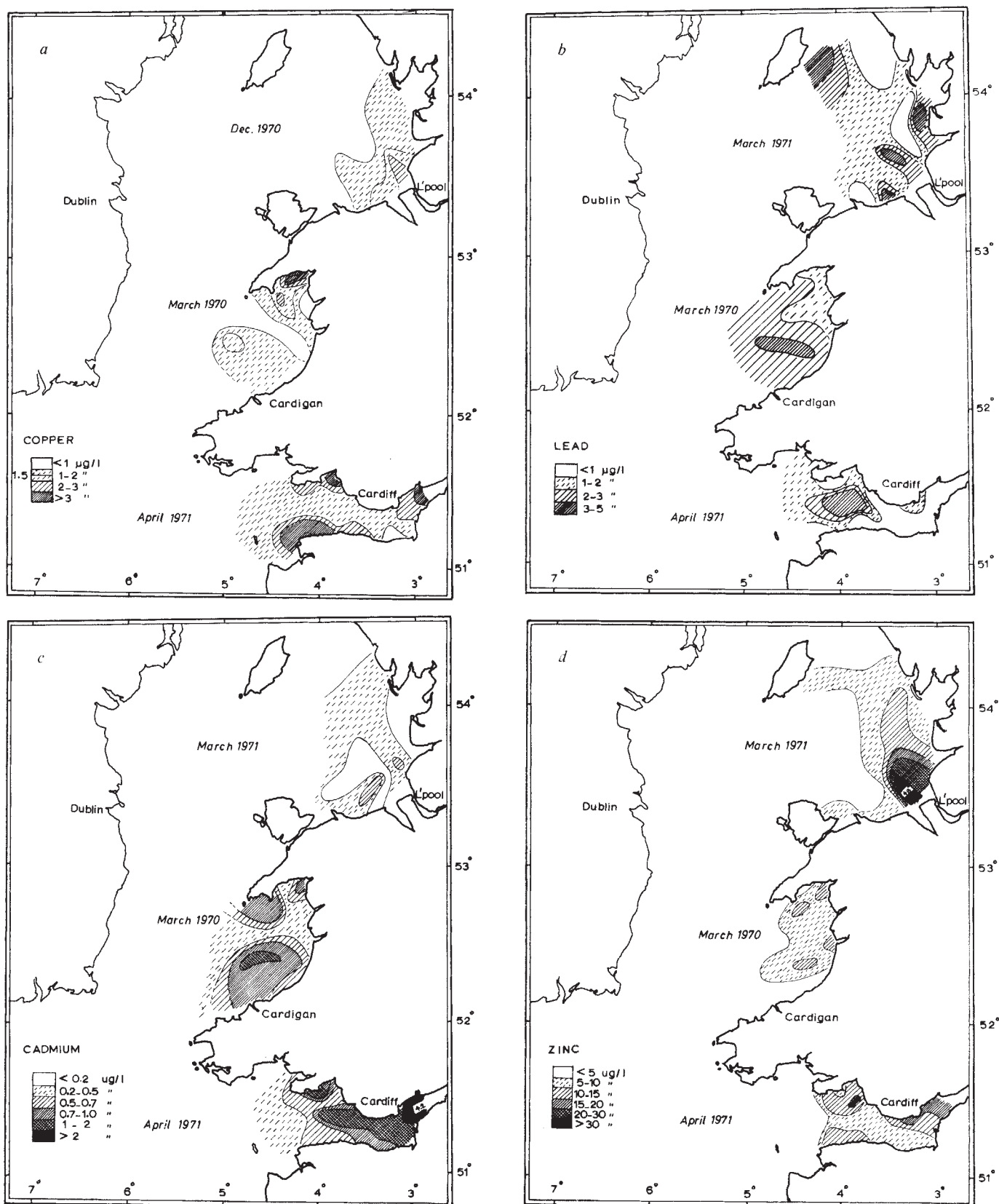


Fig. 1 The distribution of copper (a), lead (b), cadmium (c) and zinc (d) in Liverpool Bay, Cardigan Bay and the Bristol Channel surface waters.

carried into and the circulation of the water in such environments. Both trace-metal-rich natural runoff and industrial and domestic waste disposal in areas of limited circulation have led to a considerable increase in the concentration of such trace metals as cadmium, lead and zinc in these regions.

Very high concentrations found over wide areas appear to be associated with industrial and domestic waste. This may have been partly caused by the poor survival of one of the natural agencies of trace metal removal and control, for example phytoplankton and zooplankton population, in heavily pol-

luted waters. A detailed account of the hydrography and chemistry of the waters in these regions, together with a study of the seasonal chemical variation, is in hand.

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Radiocarbon Dating of Parchment

A NUMBER of parchment manuscripts of known historical age, from the period of the Renaissance, have been measured by the radiocarbon method to assess the feasibility and accuracy with which such documents can be dated. This study was also intended to check the veracity of secular variations in the radiocarbon content of the biosphere with animal rather than tree ring tissue.

time after manufacture, so that for the practical purposes of radiocarbon dating manufacture and writing are determined simultaneously. With increasing consumption, however, inadequate supply and consequently rising prices meant that some parchment documents were re-used. Their original text was erased and removed by washing. Such re-used parchments have been called palimpsests, and can be recognized by chemical and ultraviolet detection techniques. In contrast to the close temporal connexion between grazing animal, freshly prepared parchment and its utilization, almost all paper manufactured before the middle of the nineteenth century was made of cotton and linen rags, often of a considerable age. Therefore, such paper would not be so suitable as parchments for controlled radiocarbon dating.

The samples dated in this study were first selected for their historical age range. Subsequently they were examined for possible palimpsest use by ultraviolet radiation which illuminates previously erased passages. No such re-used documents were encountered. To obtain datable substance the borders of each document were cut off, preserving the text. Because the manufacture of parchment involves treatment with lime and chalk, all specimens were treated thoroughly with cold dilute hydrochloric acid, washed repeatedly with distilled water and dried. Then the samples were burnt to CO₂ which was extensively purified to remove electro-negative impurities, and finally assayed in a proportional counter for radiocarbon³. An aliquot of each gas sample was analysed for its ¹³C/¹²C isotope ratio, expressed in per mil deviation from the PDB standard. The results of the analyses are given in Table 1.

It is interesting that the radiocarbon dates after correction and calibration for secular variations correspond to their known historical ages. But the nature of the calibration curve

Table 1 Comparison of Historical and Calibrated Radiocarbon Ages of Parchments

Lab. No. UCLA	Nature of sample (g)	Quantity of sample (g)	¹³ C/ ¹² C ratio (PDB)*	Corrected ¹⁴ C age† <i>t</i> _{1/2} = 5,568 (yr)	Calibrated ‡ ¹⁴ C date (yr AD)	Historical date, AD
1441	Land lease	11.4	-22.48	100 ± 50	1820 ± 40, 1720 ± 30 or 1650 ± 15	1788
1442	Instr. of sasine	12.5	-22.31	85 ± 50	1750 ± 20 or 1680 ± 15	1752
1443	Land lease	6.4	-18.24	170 ± 50	1650 ± 15	1666
1445	Land lease	4.9	-21.37	245 ± 50	1600 ± 30 or 1500 ± 25	1495
1447	Land sale	4.7	-24.79	245 ± 50		1579
1448	Lease transfer	9.4	-21.44	250 ± 50		1578

* Compared with the PDB (Peedee Belemnite); standard of H. Craig.

† Includes ¹³C/¹²C isotopic ratio correction by standard method.

‡ Alternate dates are due to nature of calibration curve as discussed in refs. 3-5.

Previously an important manuscript, the Book of Isaiah of the original Dead Sea scrolls, had been dated by Libby *et al.*¹. This manuscript was written on heavy parchment, but direct dating was not carried out because of its great historical and religious value. Instead the linen wrappings were dated to be 1,917 ± 200 yr old (C-576) which is taken as confirming the antiquity of the document itself.

For the present experiment a number of legal documents written on parchment were obtained from England. These indentures show in writing their dates of execution ranging from the late fifteenth to the end of the eighteenth centuries.

In general, parchment is composed of specially prepared skin of sheep, ewes or lambs. Calf skin from very young or stillborn calves is referred to as vellum. The most common manufacturing process involves fleecing, polishing by scraping, lime treatments, stretching and finally drying². From roughly the fifth to the fifteenth centuries almost all documents, and from the eighth charters especially, were written on parchment. This was used for writing within relatively short periods of

first developed by Suess⁴ sometimes permits age ranges or alternative dates rather than unique dates. Consequently, for samples of unknown age it may be necessary to use independent criteria to narrow the choice. The best periods for dating in the Middle Ages are the thirteenth and fifteenth centuries.

From a geochemical point of view the parchments dated show the same deviations from the ideal constant production rate of ¹⁴C as do dendrochronologically dated tree rings. This corroborates the course of the calibration curve developed by Suess⁴ and those portions checked by others⁵. Such a result was to be anticipated for well mixed radiocarbon concentrations as observed in the general biosphere containing animals and plants alike. But this study strengthens the expectation that movement of tree sap or resin across the tree rings used for defining the calibration curves^{4,5} is not a significant factor for recent centuries.

The possibilities opened up through this investigation should permit a closer examination of such documents as the Vinland Map.

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BIOLOGICAL SCIENCES

α -Foetoprotein in Normal Human Serum

α -FOETOPROTEIN (α -FP) has been considered specific for the foetal and neonatal periods^{1,2} and for certain types of malignant tumours^{3,4}. The occurrence of α -FP in viral hepatitis has recently been reported^{5,6}. The presence of α -FP in malignant disease is usually thought to result from derepression of a foetal gene that is normally suppressed at the adult stage. We now report the detection of α -FP in sera of healthy non-pregnant human adults. The presence of α -FP in adult sera was demonstrated by radioimmunoassay (RIA) and confirmed by RIA of concentrated α -FP preparations from normal human serum using immunoadsorbents.

Anti- α -FP serum was prepared in sheep⁷. α -FP was purified from an α -FP-anti- α -FP immunoprecipitate, which was dissolved at pH 2.5 and α -FP was separated from the antibody on a 'Sephadex G-200' column⁷. The purified α -FP was radioiodinated by the chloramine-T method⁸. The labelled α -FP was separated from unreacted ¹²⁵I by gel filtration on 'Sephadex G-25', after which 98% of the label was attached to α -FP. In radioimmunoassay, free and antibody-bound labelled α -foetoproteins were separated from each other by anti-sheep- γ -globulin⁹. Of the labelled protein, 70% was bound in antibody excess. The antiserum was used at a final dilution of 1 : 600,000, where 20% of the labelled protein was bound. The standard curve covered a range from 0.25 to 25.0 ng of unlabelled α -FP. Dilutions were made in a 1 : 50 dilution of a normal human serum from which α -FP had been removed by absorption with insolubilized anti- α -FP serum (protein diluent). The adult sera were diluted 1 : 5 or 1 : 50 for testing in RIA.

In RIA none of the normal human sera tested inhibited at a dilution of 1 : 50, but were all inhibited when diluted 1 : 5. The calculated α -FP concentrations of these sera varied from 4.0 to 10.5 ng/ml. (Table 1). The α -FP concentration of foetal serum is at its highest about 2.8 mg/ml.², and it may be as high as 71 mg/ml. in the serum of hepatoma patients¹⁰.

As the RIA may not give reliable quantitative results at the

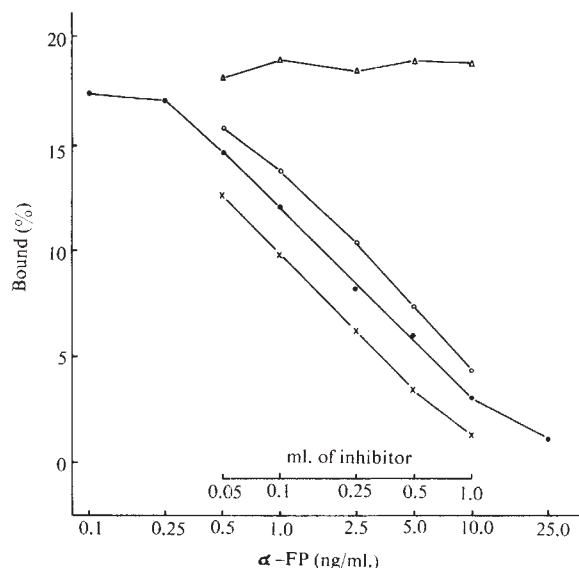


Fig. 1 Radioimmunoassay of α -FP. Standard inhibition curve was produced using 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 25.0 ng of α -FP (●) (weight scale). The three other curves show the inhibition given by serial dilutions of eluates from immunoadsorbents treated with PBS (Δ), normal human serum (NHS) ($\circ$), or diluted cord serum ($\times$) (volume scale).

high serum concentrations used, an anti- α -FP immunoadsorbent was used to confirm the presence of α -FP in normal human serum. Fifty ml. of a 1 : 3 dilution of normal human serum, 50 ml. of a 1 : 5,000 dilution of a cord serum and 50 ml. of phosphate buffered saline, pH 7.4 (PBS) were each mixed with glutaraldehyde-insolubilized anti- α -FP serum¹¹. The amount of the immunosorbent was adjusted to remove 80% of the α -FP reactivity from diluted cord serum containing 20 ng/ml. of α -FP. After overnight incubation with mixing, the immunoadsorbents were washed and eluted with 4 ml. 0.033 M citrate buffer, pH 2.5. Protein diluent (100 μ l.) was added to the eluates, which were subsequently dialysed against distilled water, lyophilized, redissolved in protein diluent and analysed for α -FP by RIA (Fig. 1). α -FP activity was recovered from the serum and cord serum-treated immunoadsorbents, whereas no α -FP activity was obtained from the PBS-treated adsorbent. Material obtained from normal adult serum, and from cord serum, gave an inhibition curve parallel to the standard curve. This is strong evidence that the inhibitory material is α -FP.

Further evidence of the existence of α -FP in normal human serum comes from electrofocusing experiments. In normal human serum the activity inhibiting in RIA was present in the fractions corresponding to the isoelectric point of the foetal α -FP⁹.

The presence of α -FP in normal human serum indicates that the suppression of the foetal gene responsible for α -FP production, although remarkably effective, is not complete at the adult stage. This is parallel to the finding by Martin and

Table 1 α -FP Concentrations of Eleven Normal Human Sera measured by Radioimmunoassay of Serum Dilutions 1 : 5

Serum	ng/ml.
1	6.2
2	7.5
3	10.0
4	10.5
5	10.5
6	6.0
7	6.2
8	4.5
9	4.0
10	7.5
11	8.5

Martin¹² that small amounts of a carcinoembryonal antigen, which has been considered specific for the foetal and cancerous colon¹³, can be demonstrated in normal adult colon. It is possible that the carcinofoetal antigens in adults are produced by relatively undifferentiated cells and that these give rise to the malignant cells. This is an alternative to the commonly held view that malignancy is associated with dedifferentiation.

As the α -FP concentration has to be 1,000 times greater than the normal level in order to be detectable by immunodiffusion, RIA of α -FP facilitates the diagnosis of primary cancer of the liver. Our preliminary findings also indicate that it might be helpful in the diagnosis of other liver diseases and some complications of pregnancy.

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Probable Genetic Linkage between Human Serum Amylase (Amy_2) and Duffy Blood Group

HUMAN serum amylase polymorphism was reported in detail by Kamarýt and Laxová¹. Two loci, with codominant alleles, control four different isoamylases in man which are detectable in serum by agar gel electrophoresis. The first locus, Amy_1 , controls serum salivary isoamylase. The two salivary alleles are Amy_1A , the most frequent in the population, and Amy_1B . A possible third allele, Amy_1C , results in the apparent silent salivary isoamylase in the serum or urine. The second locus, Amy_2 , controls serum pancreatic isoamylase. The two pancreatic alleles are Amy_2A , the most frequently observed, and Amy_2B . A search for linkage between Amy_2 and Duffy (Fy) was suggested by the finding of probable linkage between Amy_2 and the uncoiler region on chromosome 1 ($UN-1$) by Kamarýt *et al.*⁶ and the previous finding of linkage between Duffy and $UN-1$ by Donahue *et al.*⁷.

Amylase phenotypes were investigated in serum samples by agar gel electrophoresis, modified from the method of Kamarýt and Laxová¹. The serum samples were obtained from family members attending the genetics clinic. The patterns observed were identical to those described by Kamarýt and Laxová¹. 2 ml. of a 1% agar solution (Oxoid 'Agar No. 2') was pipetted onto a 76 mm $\times$ 26 mm microscope slide. Veronal buffer pH 8.4, ionic strength 0.05) was used in the gel and in the

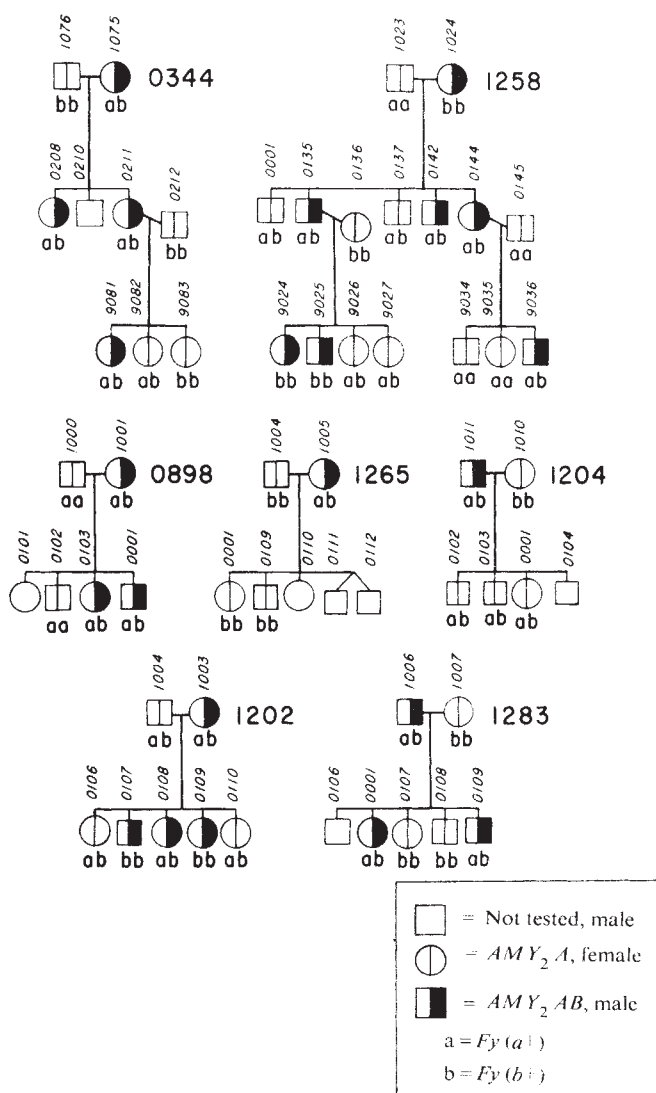


Fig. 1 Segregation of alleles at the Amy_2 and Fy loci in nine informative matings from seven UOMS families.

electrode compartments. A 1 : 1 solution of 2% agar and serum was added to slots located 15 mm from the anodic end of the slide. A stabilized current gradient of 5.5 milliamps per slide was applied for a period of 2 h at room temperature.

Zones of amylase activity were detected by sandwicheing the agar with starch slides following electrophoresis². Glass slides were coated with a 1% starch-agar solution made with a phosphate buffer (pH 5, 0.05 ionic strength) and then allowed to dry. Following an incubation period of 1 h at 37° C, the slides were immersed in a I_2 .KI solution until transparent zones of amylolytic activity were revealed against the dark blue background. The Duffy antiserum, used for the blood grouping, was prepared in our own laboratory. Uncertain test results were confirmed with antisera from commercial sources. The anti-Duffy (a) serum was a pooled, ABO absorbed serum that gives a grade ++ or +++ antiglobulin reaction after 30–60 min incubation at 37° C. The anti-Duffy (b) serum was a single, group B absorbed serum that gives a grade ++ antiglobulin reaction after equivalent incubation. The test procedure followed the standard antiglobulin (Coombs) test method.

Nine matings from seven kindreds were informative for genetic recombination between the Amy_2 and the Fy loci (Fig. 1). Each of these matings represents a double backcross between the Amy_2 and Fy loci except family 1202 which is a backcross–intercross mating. From the present data, twenty-

Table 1 Lod Scores for Recombination between *Amy*₂ and *Fy*

Family No.	Parents		Scorable offspring	Recombination fraction, θ									
	Father	Mother		0.00	0.01	0.02	0.03	0.04	0.05	0.1	0.2	0.3	0.4
0344	1076	1075	2	0.301	0.292	0.284	0.275	0.266	0.258	0.215	0.133	0.064	0.017
0344	0112	0111	3	— ∞	—1.106	—0.813	—0.646	—0.530	—0.442	—0.188	0.010	0.070	0.061
1258	0135	0136	4	1.204	1.187	1.169	1.151	1.133	1.115	1.021	0.816	0.584	0.237
1258	0145	0144	3	0.903	0.890	0.877	0.863	0.850	0.836	0.766	0.612	0.438	0.237
0898	1000	1001	3	0.602	0.588	0.576	0.562	0.549	0.535	0.465	0.318	0.170	0.049
1265	1004	1005	2	0.301	0.292	0.284	0.275	0.266	0.258	0.215	0.133	0.064	0.017
1204	1011	1010	3	0.602	0.588	0.576	0.562	0.549	0.535	0.465	0.318	0.170	0.049
1202	1004	1003	2	0.301	0.292	0.284	0.257	0.266	0.258	0.215	0.133	0.064	0.017
1283	1006	1007	4	0.903	0.886	0.868	0.850	0.832	0.814	0.720	0.517	0.298	0.094
Total			26	— ∞	3.911	4.105	4.167	4.181	4.167	3.894	2.990	1.922	0.858
				0	8,147	12,735	14,690	15,170	14,689	7,834	977	83	7
												Z (lod score)	(λ) antilog

six offspring are informative and phase is known for ten individuals in families 0344 and 1258. These include one recombinant and nine nonrecombinants. Without knowledge of phase, individuals 0106, 0108 and 0110 in family 1202 cannot be scored. The remaining sixteen scorable individuals are probably nonrecombinants. Segregation at each locus does not differ from the expected of thirteen; there are twelve *Amy*₂*AB* ($\chi^2 = 0.077$; $0.70 < P < 0.80$) and fifteen *Fy* ($a+b+$) ($\chi^2 = 0.31$; $0.50 < P < 0.70$). Morton's lod (logarithm of the odds) scores have been calculated (Table 1) as described by C. A. B. Smith³. The maximum lod score is 4.181 at a recombination fraction, θ , of 0.04 with a likelihood (antilog) of 15,170 (Fig. 2). The averaged likelihood, Λ calculated according to the method of C. A. B. Smith⁴, is 2,994. Therefore, the probability of linkage,

$$\frac{\Lambda}{\Lambda + 17.5} = 0.9941$$

17.5/1 is the initial probability⁵. The 95% confidence limits for θ are 0.02–0.22.

Three additional matings were ascertained in which the silent allele, *Amy*₁*C*, was present. In each instance free recombination was demonstrated between *Amy*₂ and *Fy*. One possible explanation is that the phenotype has been improperly designated and what has been referred to as *Amy*₁*C* is actually a faster moving *Amy*₁*A* band that is observed in the *Amy*₂ region following electrophoresis. Investigation of the amylase variants by biochemical methods is proceeding in order to investigate the nature of the *Amy*₁*C* phenotype.

Kamarýt, Adámek and Vrba⁶ suggested the possibility of close linkage between the *Amy*₂ locus and the uncoiler region on chromosome 1 (*UN-I*). Donahue *et al.*⁷ demonstrated

localization of the Duffy locus to a region within mappable distance of *UN-I*. Therefore this present preliminary report indicates that the *Amy*₂ locus, since it is linked to Duffy, is also on chromosome 1, near *UN-I*. The gene order could be either *UN-I-Amy*₂-*Fy* or *UN-I-Fy-Amy*₂. Further investigation is being conducted in families segregating for *UN-I* as well as *Amy*₂ and *Fy* so that gene order may be established.

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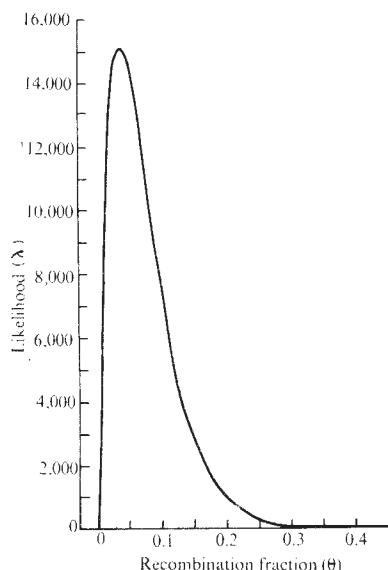


Fig. 2 Likelihood for a recombination fraction (θ) between *Amy*₂ and *Fy*.

Antagonism of Cholera Enterotoxin by Anti-inflammatory Agents in the Rat

THE treatment of cholera by intravenous fluids and electrolytes is well established and, if instituted before dehydration and shock are irreversible, can completely protect the patient. But in times of large-scale outbreaks neither the personnel nor material (approximately 10–20 l. per patient) is usually available¹. Trials of oral fluid and electrolyte replacement therapy have been effective because the absorption of salts, water and carbohydrates are unimpaired by such treatment^{2–4}. This greatly simplifies treatment, but again limitation in the availability of materials and personnel may not allow treatment of all victims during severe epidemics. Since both these methods do not treat the severe diarrhoea but only attempt to replace fluid and electrolyte losses throughout the period (3–5 days) of diarrhoea, the patient is still debilitated and unable to carry on normal activities. A drug which could be given orally to reverse the massive loss of fluid and electrolytes by antagonizing the effect of cholera enterotoxin on secretory mechanisms of the small intestine has been suggested as a rational means to attack cholera with the least number of trained people and equipment^{5–7}.

Although several animal models for studying the activity of cholera enterotoxin have been described using dogs⁸⁻¹⁰, rabbits¹¹ and rats¹², a test which is more suitable for the rapid testing of drugs has been devised and used to study several compounds.

Female Holtzman rats weighing 85-120 g (heavier rats are less sensitive) are fasted for 18 h and anaesthetized with 25 mg/kg, given intraperitoneally, of sodium methohexital. A midline incision is made and a cotton ligature is placed and tied at the ileocaecal junction. Substances injected intraduodenally are all given in a volume of 1 ml. using a 26-gauge needle. The midline incision is closed with wound clips and the rats are placed in plastic boxes to recover from anaesthesia. Four hours after ligation the rats are killed with chloroform, the incision is reopened and a ligature is placed around the oesophagus. The stomach and small intestine are removed carefully and weighed to the closest 0.1 g. Since the weight of the stomach and small intestine varies with the weight of the rat, all weights are stated as g of gut/100 g rat.

There was no significant difference at 4 h in gut weight between rats administered 1 ml. of saline with or without the ileum ligated. Intraduodenal administration of 10-80 mg of crude lyophilized cholera toxin (from Dr John Seal, National Institute of Allergy and Infectious Diseases, made by the method of Finkelstein *et al.*¹³) produced a significant increase in gut weight which was dose-related, but cholera toxin (40 mg/ml.) boiled for 1 h produced no change (Table 1).

Table 1 Effect of Cholera Toxin on Fluid Accumulation in 4 h Ileal-ligated Rats

Group	Dose	N	Body weight (g) mean $\pm$ s.d.	Gut weight† g/100 g rat mean $\pm$ s.d.
Saline	1 ml.	10	109.4 $\pm$ 6.9	7.3 $\pm$ 0.6
Inactivated toxin	40 mg/ml.	10	108.7 $\pm$ 7.0	7.6 $\pm$ 0.8
Toxin	10 mg/ml.	10	106.5 $\pm$ 8.7	8.3 $\pm$ 0.8*
Toxin	20 mg/ml.	10	106.3 $\pm$ 7.9	8.6 $\pm$ 0.8*
Toxin	40 mg/ml.	10	110.1 $\pm$ 3.2	9.8 $\pm$ 0.9*
Toxin	80 mg/ml.	9	106.7 $\pm$ 5.9	10.2 $\pm$ 1.8*

*Statistically significant difference at $P=0.05$ level from saline and inactivated cholera toxin controls using analysis of variance.

†Tissue and fluid.

Drugs examined for anticholera activity were either administered orally 30 min before ligation or subcutaneously at the time of ligation. Subcutaneous administration was preferred since it avoided the osmotic accumulation of fluid, but oral administration was also used since it is the preferred clinical route.

Several anti-inflammatory agents were tested since cholera toxin induces swelling of the feet in rats⁶ which is blocked by indomethacin⁷. Ethacrynic acid was tested because this compound is reported to be effective against cholera toxin in dogs and rabbits¹⁴.

Anti-inflammatory compounds inhibited cholera toxin-induced accumulation of fluid and in the higher doses decreased gut weight in rats given only saline; both effects were dose-related (Table 2). The steroid anti-inflammatory agents dexamethasone and prednisolone were effective in relatively high doses. Of the nonsteroids tested, indomethacin was the most active, showing activity at doses as low as 0.06 mg/kg given subcutaneously. Aspirin, indomethacin and phenylbutazone were also active orally. Ethacrynic acid, which has minimal diuretic activity in rats, was active against cholera toxin at a dose of 32 mg/kg given subcutaneously but was not active orally, unlike the other compounds tested in that it did not seem to affect the weight of the gut in rats given only saline.

The doses of nonsteroids required for activity against cholera toxin in the rat seem to be in the same range as those for anti-inflammatory activity¹⁵. The single doses of steroids required for effect are high, but they are comparable with doses used acutely in patients with severe shock.

Table 2 Effect of Compounds on Cholera Toxin-induced Fluid Accumulation in Ileal-ligated Rats

Compound	Dose (mg/kg)	Route	% of saline control*	% Inhibition of cholera toxin (40 mg) ††
Aspirin	6.25	S.C.	104	14
	25.0	S.C.	102	55
	50.0	S.C.	87	99
	100.0	S.C.	91	100
	50	P.O.	81	45
	100	P.O.	81	90
Indomethacin	0.06	S.C.	104	30
	0.125	S.C.	108	49
	0.5	S.C.	87	51
	1.0	S.C.	86	59
	1.0	P.O.	91	77
	4.0	P.O.	78	78
Phenylbutazone	16.0	P.O.	82	100
	8	S.C.	93	24
	16	S.C.	81	43
Sodium salicylate	16	P.O.	81	68
	50	S.C.	93	39
Dexamethasone	2	S.C.	88	55
	4	S.C.	89	76
	8	S.C.	86	88
Prednisolone	1	S.C.	95	0
	2	S.C.	90	9
	4	S.C.	89	92
Ethacrynic acid	32	S.C.	97	55
	32	P.O.	112	11

*Percentage of saline control =

$$\frac{\text{gut weight of drug-treated rat}}{\text{gut weight of saline-treated rat}} \times 100$$

$$\dagger \text{Percentage inhibition of cholera toxin} = \frac{\Delta C - \Delta d}{\Delta C} \times 100$$

ΔC = (gut weight of rats receiving cholera) — (gut weight of rats receiving saline).

Δd = (gut weight of rats receiving cholera toxin + drug) — (gut weight of rats receiving saline + drug).

††Given intraduodenally.

The outpouring of fluid and electrolytes into the small intestine in cholera seems to be due to stimulation of secretion and not to interference with sodium or glucose absorptive capacity¹⁶. The mechanism for this activity is not clear, but several naturally occurring substances have been shown to produce similar effects. Both cyclic AMP and prostaglandin E_1 stimulate intestinal secretion but they are less potent than cholera enterotoxin and act more effectively by intra-arterial rather than by luminal administration¹⁷.

The compounds active against cholera toxin in our test may act at several sites. They may inhibit secretory processes or stimulate absorptive mechanisms, they may block receptor sites for the toxin, or they may alter its detoxification. Recent studies have shown that¹⁸ aspirin and indomethacin inhibit the synthesis of $PGF_{2\alpha}$ from arachidonic acid and reduce prostaglandin released from the dog spleen¹⁹. Bennett has suggested that cholera toxin stimulates the release or formation of prostaglandin at intramural sites and that this may be blocked by indomethacin- or aspirin-inhibition of prostaglandin synthesis²⁰. Whether these drugs are effective in doses that could be tolerated by cholera victims is not yet known.

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Studies on the Biogenesis of Herpesvirus Envelope

THE nucleocapsid of the herpesvirus is surrounded by an envelope which the virion acquires by budding from the nuclear membrane of the infected cell¹⁻⁴. The viral envelope, however, contains glycoproteins which differ in their pattern of migration on acrylamide gels from those normally present in the nuclear membrane⁶. Proteins associated with the nuclear membrane at the time of infection do not become part of the virions, even though these proteins are not lost from the membrane after infection refs. 5-7 and (unpublished results).

Whereas the viral envelope contains proteins synthesized by the cells only after infection, its phospholipids are chiefly those pre-existing at the time of infection^{5,8}. Nuclear membrane phospholipids in regions from which the virus buds have, however, in certain conditions of labelling, a specific activity different from that of the phospholipids in the remainder of the nuclear membrane⁵. These findings indicated that infection may stimulate the growth of the nuclear membrane, but that the phospholipids which become part of growing areas of the nuclear membrane are derived from other cellular membranes. We have investigated whether a virus-induced flow of phospholipids from the cytoplasmic to the nuclear membranes can be detected.

Primary rabbit kidney cells were cultivated as described previously⁹, and infected with pseudorabies virus¹⁰ (one of the herpesviruses) with an adsorbed multiplicity of 20 plaque-forming units (p.f.u.)/cell. The cells were separated into fractions containing the cytoplasmic, outer nuclear, and inner nuclear membranes by the procedure described in Table 1. As choline becomes part of membrane-associated phospholipids only¹¹, we refer to the phospholipids extracted from the different fractions (after extensive acid washing) as being from the membranes present in each of the fractions.

The phospholipids in the membranes of mammalian cells have a complex pattern of renewal which differs for each of the membranes associated with various subcellular fractions^{5,12-16}. Preliminary experiments were therefore necessary to determine the distribution of labelled phospholipids among the cytoplasmic and nuclear membranes at various times after a "chase" (Table 1).

After a 3 day chase there was a loss of 42% of labelled phospholipids from the cytoplasmic fraction and an approxi-

Table 1 Distribution of Label derived from ³H-Choline among the Phospholipids of the Cytoplasmic and Nuclear Membranes at Different Times after Removal of Labelled Medium

Time (days)	No. of cells/culture × 10 ⁻⁶	Type of membrane		
		Cytoplasmic	Outer Nuclear	Inner
0	2.1	19,950 *	1,650	760
1	3.1	14,100	1,500	1,180
2	3.8	12,300	1,430	1,310
3	3.9	11,500	1,320	1,480

* c.p.m./culture.

Monolayers of primary RK cells, 3 days after seeding, were incubated in Eagle's synthetic medium containing 3% dialysed bovine serum and 0.2 μCi ³H-choline/ml. for 72 h during which the number of cells per culture increased approximately five times. The cultures were then washed six times with unlabelled medium and further incubated in unlabelled medium (start of chase). At daily intervals, cells were scraped into RSB at a concentration of 4 × 10⁶ cells/ml. and the cytoplasmic fraction was separated from the nuclear fraction according to Penman¹⁷. The pellet containing the nuclei was treated with 0.5% 'Triton X-100' to remove the outer nuclear membrane¹⁸ and adherent cytoplasmic tags. Since phospholipids are associated only with membranes, the membranes in the cytoplasmic and nuclear fractions were not further purified. Instead, each of the fractions was washed with cold perchloric acid, 0.2 N, and the radioactivity in the lipid fractions was determined as described elsewhere⁵.

mate doubling in the inner nuclear fraction. (Further incubation of the cells in unlabelled medium did not affect significantly the distribution of labelled phospholipids in these fractions.) The outer nuclear membrane fraction did not change significantly throughout the experiment and it is probable that this fraction was contaminated with cytoplasmic membranes.

The increased amount of labelled phospholipid associated with the nuclear membrane fraction could be due to the increase in the number of cells in the culture, as the number did increase during the chase period. However, the labelled phospholipids associated with the nuclear membrane fraction increased when there was a decrease in labelled phospholipids associated with the cytoplasmic membranes, indicating that a precursor relationship between the phospholipids present in different cellular membranes may exist.

The results obtained when cultures are infected after a 3 day chase period (in an experiment similar to that described in Table 1) are summarized in Table 2. A virus-induced increase in the amount of label associated with the inner nuclear membrane was observed; this increase was substantial 4.5 h after infection (70%) and reached a maximum 7 h after infection (98%). Beyond 8 h, the amount of label associated with the nuclei decreased. At late stages of infection, however, the nuclear membrane projects outward into the cytoplasm and may become detached⁴; nucleocapsids emerge from the nuclei and thereby remove nuclear membrane, and an increase in the fragility of the nuclei occurs. Any or all of these may

Table 2 Transfer of Phospholipids from Cytoplasmic to Nuclear Membranes after Infection with Pseudorabies Virus

Experiment	Time after infection (h)		Type of membrane		
			Cytoplasmic	Outer Nuclear	Inner
1	4.5	Infected	25,600 *	3,560	5,820
		Uninfected	26,100	3,120	3,430
2	7	Infected	29,900	6,780	5,980
		Uninfected	33,500	3,890	3,020

* c.p.m./culture.

Primary RK monolayers labelled with ³H-choline as described in Table 1 were infected or were mock-infected 3 days after the beginning of the chase period. At different times after infection, depending upon the experiment, the cells were harvested and the radioactivity in the phospholipids of the nuclear and cytoplasmic membranes was determined as described in Table 1.

Table 3 Distribution of Label derived from ^3H -Choline in Phospholipids of the Membranes from Infected and Uninfected Cells

	Type of membranes	Lysolecithin	Sphingomyelin	Phosphatidylcholine
Infected	Cytoplasmic	3.8 *	17.2	79.0
	Inner nuclear	1.3	45.2	53.5
Uninfected	Cytoplasmic	2.9	17.9	79.2
	Inner nuclear	1.5	44.5	54.0

* % of total radioactivity.

The separation of the phospholipids was performed by thin layer chromatography as described previously⁵. This table summarizes the results obtained with the fractions from Table 2, experiment 2.

contribute to a loss of nuclear membrane at late stages of infection.

The virus-induced increase in the amount of labelled phospholipids associated with the inner nuclear membrane suggests that infection stimulates the synthesis of nuclear membrane. This increase could also be due, however, to a virus-induced change in the sedimentation characteristics of cellular organelles which may result in a contamination of the nuclei with cytoplasmic membranes. As the inner nuclear membrane differs in its phospholipid composition from that of the cytoplasmic membranes⁵, we determined the distribution of label in the various phospholipids present in the nuclear and cytoplasmic membrane fractions of infected and uninfected cells. Table 3 shows that the ratio of label in sphingomyelin to label in phosphatidylcholine in the nuclear membranes of infected and uninfected cells was the same and was significantly different from the ratio found in the cytoplasmic membranes. It is clear therefore that the nuclear membrane fraction obtained from infected cells was not contaminated with cytoplasmic membranes, because, if it were, the ratio of sphingomyelin to phosphatidylcholine in that fraction would have changed.

The increased amount of labelled phospholipids associated with the nuclear membranes in the infected cells probably represents formation of new nuclear membrane. To ascertain the amount of new membrane that the approximate doubling of the labelled phospholipids associated with the nuclear membrane actually represents, it was necessary to determine the specific activity of the phospholipids of the nuclear and cytoplasmic membranes immediately before infection. Table 4 shows that the specific activities of the phospholipids associated with the two types of membrane were approximately the same. Thus, if the phospholipids that were transferred to the nuclear membrane come from a random population of cytoplasmic phospholipid molecules, the doubling (7 h after infection) of radioactivity associated with the nuclear membrane represents a doubling of this membrane.

These results show that there is in pseudorabies virus-infected cells an extensive *de novo* assembly of nuclear membrane. The phospholipids that become associated with the new nuclear

membrane are derived in most part from other cellular membranes. Since cell-specific protein synthesis is inhibited in these cells and only virus-specific proteins are synthesized, the proteins used to build the nascent nuclear membrane must be virus-specific exclusively. The virus probably buds only from the newly synthesized regions of the nuclear membrane, ensuring the presence of virus-specific proteins in the viral envelope.

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X-ray Diffraction and Fine Structural Studies of Crystals in the Malpighian Tubules of Silkworms

THE Malpighian tubules of insects serve as an excretory organ and also as a site for the storage of waste products. In the silkworm (*Bombyx mori*), these tubules are filled with birefringent crystals (Fig. 1). Early workers observed particles in Malpighian tubules and noted their appearance in the lumen during the moulting cycle^{1,2}. They tentatively identified the particles as urates, carbonates or calcium oxalate^{3,4}. Using powder X-ray diffraction, we have established that the crystals are calcium oxalate monohydrate.

Malpighian tubules were removed from larvae and dried, ground and packed in capillary tubes for powder X-ray diffraction. Exposure time was 12 h using a copper target and nickel filter ($K\alpha$ -1.54). The diffraction patterns and d-spacings of silkworm crystals were compared with known calcium oxalate and published data⁵ (Table 1).

Crystals in the cocoons of the tent caterpillar also are calcium oxalate⁶ but no crystals have been observed in the cocoons of silkworms. The crystals have solubility properties characteristic of calcium oxalate⁷.

Crystal development was investigated using electron microscopy. The tubules are one cell thick and three or more cells form the circumference, surrounding a lumen filled with crystals. Fig. 2 shows a cross section of a tubule. Around

Table 4 Specific Activity of the Phospholipids associated with the Cytoplasmic and Nuclear Membranes from Uninfected Cells

Membrane	$\mu\text{g P/ml.}$	c.p.m./ml.	c.p.m./ $\mu\text{g P}$
Cytoplasmic	42.0	59,420	1,410
Outer nuclear	4.0	6,480	1,618
Inner nuclear	4.8	7,280	1,515

RK cells, 3 days after seeding, were incubated for 72 h in Eagle's synthetic medium containing 3% dialysed serum and 0.2 $\mu\text{Ci/ml.}$ ^3H -choline. The cultures were washed six times with unlabelled medium and incubated further in the unlabelled medium for 3 days. Twenty monolayer cultures (4×10^6 cells/culture) were harvested, the cytoplasmic fractions were separated from the nuclear fractions, and the phospholipids were extracted, as described in Table 1. The amounts of phosphorus and of radioactivity associated with the phospholipids were determined as described previously⁵.

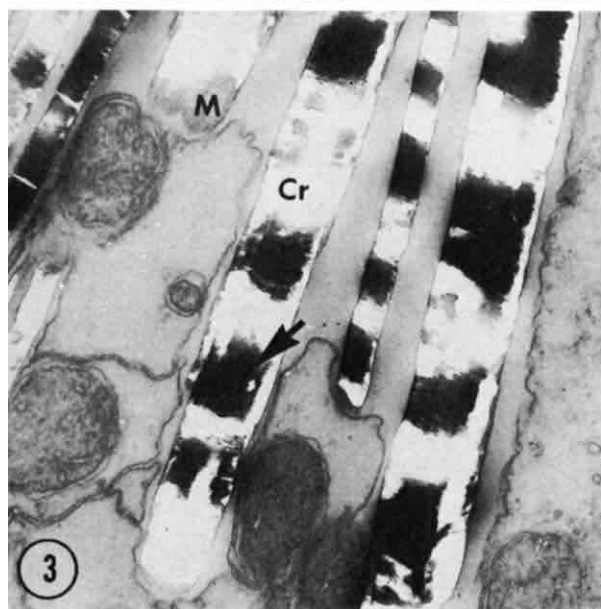
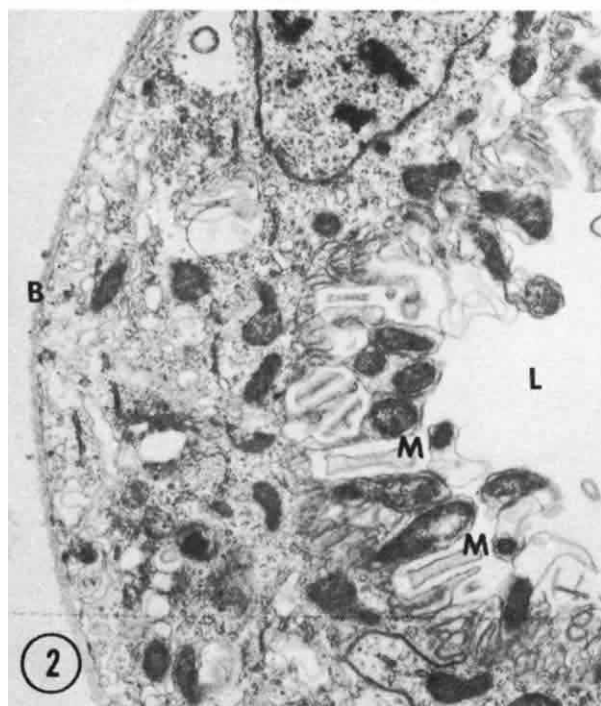
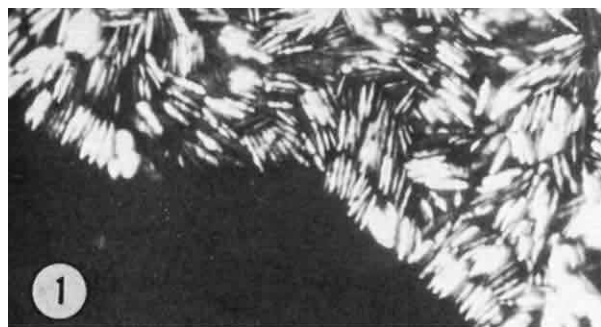


Fig. 1 Light micrograph of a longitudinal section of Malpighian tubule taken with polarized light to show the birefringence and orientation of the crystals within the tubule ($\times 600$).

Fig. 2 Electron micrograph of a cross section of a Malpighian tubule in an early stage of crystal formation. Notice the general cell features and the matrix material. B, Basement membrane; L, lumen; M, matrix ($\times 12,000$).

Fig. 3 Electron micrograph of several mature crystals. Dark material is crystalline calcium oxalate (arrow). Note the matrix material (M) that remains ($\times 26,000$).

Table 1 X-ray Powder Diffraction Data

Calcium oxalate monohydrate ^{5,6}		Malpighian tubule crystals from <i>Bombyx mori</i>	
d(A)	I/I ₁	d(A)	I/I ₁
5.89	100	5.94	100
3.65	100	3.67	80
2.95	100	2.97	80
2.49	40	2.49	40
2.33	80	2.35	60
2.25	20	2.27	20
1.94	60	1.93	40
1.73	20	1.73	20

the outside of the tubule is the basement membrane and the cell surface toward the lumen is covered with microvilli while the membrane of the opposite side is involuted. Many mitochondria are seen in all parts of these excretory cells.

At ecdysis the tubules empty of crystals and new crystals begin to form a few hours later. The first stage is the appearance in the lumen of patches of fibrillar material closely associated with the microvilli (Fig. 2), sometimes seeming to be attached to the surface of the cell membrane. This material apparently acts as a matrix in which the crystals arise. Gradually more patches of matrix appear and regions of crystalline calcium oxalate are found developing within the matrix. Finally, the crystal matrix seems to be entirely replaced (or modified) by the calcium oxalate crystals. Some matrix remains visible around each crystal and an envelope of non-crystalline material surrounds mature crystals.

During standard preparation for electron microscopy, the crystalline material is removed from thin sections, but some calcium oxalate is retained in unstained sections (Fig. 3, arrow). The accumulation of crystals in Malpighian tubules is probably a mechanism for the removal of excess oxalate present in the silkworms' diet of mulberry leaves.

A detailed report on the fine structure and relationships of the Malpighian tubule cells to these crystals is in preparation. Special acknowledgement is given to Dr H. Steinfink for making available his X-ray diffraction equipment. This work was supported by grants from NIH and the Graduate School, the University of Texas.

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Mechanism of Abdominal Extension during Oviposition in *Locusta*

THE adult female of *Locusta migratoria* digs a hole about 8–9 cm deep in sand in which to lay her eggs. The normal length of the ovipositing part of the abdomen is about 2.5 cm. Part of the increase in length is achieved by unfolding the telescoped intersegmental membranes, giving a two-fold

extension. The rest of the increase is achieved by stretching a thickened part of the intersegmental membrane¹ (Fig. 1A and B) by up to ten times the unstretched length. The possible mechanisms involved in stretching the abdomen, and some of the mechanical properties which might be expected of the intersegmental membrane if the proposed mechanism for extension is correct, are discussed.

Since the observations and illustrations of Vosseler² showing the adult female locust abdomen during oviposition (Fig. 1A), two main theories have been proposed for the mechanism of extension of the abdomen: (a) internal pressure³, and (b) the pulling by the ovipositor valves as they dig the hole⁴. (There is no morphological basis for a theory of muscular extension of the membrane at the segmental level.) But there has been little experimental evidence to support either of these theories. Not surprisingly, if the internal pressure of the female locust is raised by pumping in either water or air, then the abdomen will elongate³. In an ovipositing female the volume of air in the tracheal system increases by about 120% measured at atmospheric pressure⁵, but this takes no account of possible compression by a postulated high internal pressure. If the female locust is deprived of sand in which to lay her eggs and, as happens in laboratory culture, hangs from the wall of the cage to oviposit, the increase in length of the abdomen is only that achieved by the unfolding of the telescoped intersegmental membranes; the membranes seem not to stretch⁶.

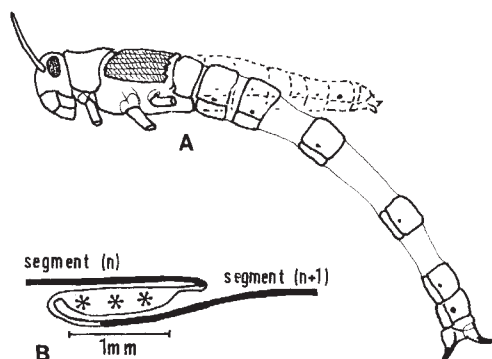


Fig. 1 A, To show the relative extensibility of the different intersegmental membranes down the abdomen. The membranes stretch more than this in life. Redrawn after Vosseler². B, Section through two sclerites (black) to show the arrangement of the extensible part (asterisked) of the intersegmental membrane in the unextended state.

To investigate the internal pressure theory a capillary tube was inserted into the mesothorax of a digging female. The result was inconclusive because the blood (which was protected from contact with the air in the capillary by a layer of liquid paraffin) does not rise as far up the tube as does water under simple capillarity. An alternative technique was to pierce the intersegmental membrane of the locust between abdominal segments 2 and 3 with a sharp razor. If the razor is sharp enough the locust does not react and the behaviour of the blood at the wound site can be observed while the locust continues to dig. At no time does blood spurt from the wound; rather the blood surface pulsates slightly in time with the ventilation cycle and movement of the first thoracic spiracle. No blood escapes, indicating that the internal pressure of the insect is no more than atmospheric, and discounting any internal pressure theories.

The abdomen must be stretched entirely by the action of the ovipositor valves pulling against the sides of the hole, and this lays constraints on the mechanical properties of the stretchable part of the intersegmental membrane.

While it is digging its hole, the locust frequently releases its grip on the walls of the hole, both for resting and for probing deeper. If the membranes were elastic, the abdomen would



Fig. 2 A female locust ovipositing in sand in a 'Perspex' observation chamber. Scale is in cm.

immediately be drawn back up the hole as the membranes contracted. In fact only a very small contraction of the membranes is noted when the insect releases its hold on the walls of the hole. Experiments in progress show that the membranes are markedly visco-elastic, showing large amounts of creep, or continued deformation under constant load; thus the membranes show marked stress-relaxation, which means that the load required to produce a given extension of the membrane falls off rapidly with time. Thus a locust can pull a membrane out to a new length, sustain the force for a short time while the membrane adapts, then release the force leaving the membrane still substantially at the new length.

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Sources of Substances which Elicit a Behavioural Response from the Insect Parasitoid, *Campoletis perdistinctus*

THERE are many reports of the competition among larval parasitoid Hymenoptera which arises when two or more eggs of the same species or of different species are deposited in the same host¹. But it may be advantageous to avoid competition, especially for those Hymenoptera which require the entire host for development. Indeed, Salt¹ has suggested that there is an advantage in a reduction of the number of progeny lost in competition within their host, for insect parasitoids can then maintain their populations with a relatively low fecundity.

To avoid competition females often select hosts which have not been parasitized previously. They do this by detecting a chemical on the host^{2,3} or the substratum⁴ or by detecting gross physical⁵ or chemical changes⁶ in the host which are presumably detected after the ovipositor has been inserted. The outcome is an abortive ovipositioned attack in which no additional egg is oviposited⁷⁻⁹. Experiments to be reported by S. B. V. have demonstrated that the ichneumonid, *Campoletis perdistinctus* (Viereck), oviposits in non-parasitized *Heliothis virescens* (Fab.) larvae in preference to superparasitized individuals. Those few ovipositional attacks on superparasitized individuals that did occur proved to be abortive in that no additional egg was oviposited by the female.

We have identified the sources of two materials which may enable *C. perdistinctus* to discriminate between parasitized and unparasitized hosts. One is an oily substance isolated from the alkaline gland (Dufour's gland) of *Campoletis* females. *H. virescens* larvae treated topically with this material were not attacked by *Campoletis*. The second material is a physiological saline-soluble fluid from the oviducts of *Campoletis*. This material injected into the haemocoel of *H. virescens* acted as an ovipositional deterrent (ovipositor was inserted but no egg was oviposited).

The *C. perdistinctus* females used in this study were maintained in the laboratory on a solution of honey and water. The host, *H. virescens*, was reared in the laboratory on artificial medium¹⁰. The reproductive system of *Campoletis* is illustrated in Fig. 1 for easy identification of the structures discussed.

The reproductive system was removed from adult females by gently grasping and pulling the last two abdominal segments and ovipositor from the rest of the body. The alkaline gland, poison gland and lateral oviduct were then separated from each other. Concentrated solutions of the contents of each organ were made by breaking open six of them in a small drop of solvent. Acetone was used to dissolve the contents of the alkaline gland and Pringle's¹¹ saline solution was used to dissolve the material from both the lateral oviduct and poison gland. Second instar *Heliothis* were treated topically by dipping them in the solution of the contents of the organ to be tested. *Heliothis* larvae dipped in the appropriate solvent were used as controls. Five controls and five treated *Heliothis* were placed on a piece of filter paper in a 10 cm Petri dish. Control larvae were marked with a small drop of water soluble dye for easy identification. A female *Campoletis* was then introduced into

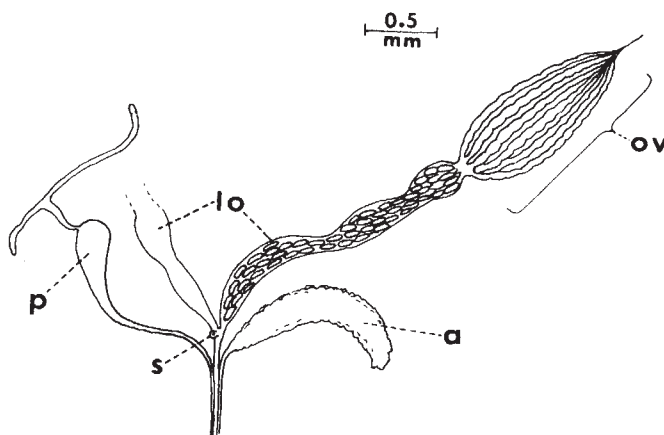


Fig. 1 Female reproductive system of *Campoletis perdistinctus*. Only the right lateral oviduct and ovary are shown. p, Poison gland; s, spermatheca; a, alkaline gland (Dufour's gland); lo, lateral oviduct; ov, ovary.

the Petri dish and her choice for oviposition recorded. The attacked individual was removed immediately afterwards and replaced with a similarly treated individual so that a 1:1 ratio was maintained at all times. This procedure was carried out until thirty-six observations (ovipositional attacks) were made. Statistical analysis was done by means of the sign test¹². A value of less than twelve is significant at the 5% level of probability. The alkaline gland solution (Table 1) was the only one of the three tested substances to show a significant degree of activity. *Campoletis* females were selected to attack the control individuals in preference to the alkaline-gland-treated individuals. There was no significant degree of selection for the control larvae over those treated with either the poison gland solution or oviduct fluid.

Third instar larvae were injected with solutions of poison gland, alkaline gland and oviduct fluid to determine if any of these was an ovipositional deterrent. A micro glass pipette, made from capillary tubes, was used to inject the test solution into *Heliothis*. The equivalent of one gland or the fluid from one oviduct was injected into each larva. The glands and oviduct were broken open in just enough solvent to make the injections of their contents into the larvae possible. Control larvae received an equivalent volume of Pringle's saline. Each group of treated larvae was immediately exposed to a *Campoletis* female and after each larva was attacked three times it was removed and dissected to determine the number of eggs oviposited. Student's *t* test was used for analysis of the results (Table 2). The oviduct fluid was the only substance tested which significantly reduced the number of eggs oviposited from that of the number oviposited in the control group. The larvae in all groups of treated animals were readily searched and attacked by *Campoletis*, but those injected with oviduct fluid were rejected after the ovipositor was inserted. An observation pertinent to this result is that *Campoletis* abruptly withdrew its ovipositor from those larvae injected with oviduct fluid.

The materials isolated from both the alkaline gland (Dufour's gland) and the oviducts may properly be called pheromones. Host discrimination by a braconid parasitoid, *Orgilus lepidus*

Table 1 Number of Topically Treated *Heliothis* Larvae attacked by *Campoletis*

Treatment with solution of	No. of attacked larvae*	
	Control	Treated
Poison gland	23	13
	17	19
Alkaline gland	33	3†
	31	5†
Lateral oviduct	22	14
	21	15

*Number of observations for each replication was thirty-six.

†Significant at 95% level of probability.

Table 2 Number of Eggs oviposited in *Heliothis*

Injection with solution of	No. of larvae	No. of ovipositional attempts/larva	Total eggs oviposited	Average No. of eggs/larva	s.e.
Poison gland	14	3	38	2.7	0.61
Alkaline gland	9	3	22	2.4	0.76
Lateral oviduct	14	3	10	0.7*	0.99
Saline	15	3	39	2.6	0.63

*Significant at 99% level of probability, Student's *t* test.

Musebeck, was also reported to result from a substance left on its host by earlier females and from the detection of an oviposition deterrent within the parasitized host¹³. The poison gland of this species was considered the source of the deterrent and not the lateral oviducts as was the case for *C. perdistinctus*. The source of the external pheromone was not definitely identified for *O. lepidus*.

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Spin Echo Studies on Cellular Water

HIGH resolution nuclear magnetic resonance (NMR) studies on rat skeletal muscle have recently shown that cellular water produces an absorption line almost ten times as broad as the line width of pure water¹. This suggests that these water molecules are at least partly bounded such that the proton correlation time is increased. Similar observations have also been made for deuterated water in skeletal muscle² and for H₂O in other tissues³⁻⁵. Our results further substantiate the notion that ordered water exists in biological tissue and rules out several criticisms of this interpretation.

To obtain more precise and detailed information about the cellular water, we have complemented the high resolution studies. The spin-lattice relaxation time (T_1), the spin-spin relaxation time (T_2) and diffusion coefficient (D) were measured by procedures given elsewhere^{6,7}. All experiments were performed at room temperature and the results are summarized in Table 1. Pure water in identical experimental conditions gave values for T_1 , T_2 , and D very close to earlier published data^{8,9}. We find $T_1/T_2=1.85$ for pure water in agreement with the observations of Meiboom *et al.*⁸.

These measurements on mammalian muscle water agree with the findings of the previous high resolution¹ and pulsed^{3,4} NMR studies. The diffusion coefficient of muscle water was also in qualitative agreement with that determined for a variety of biological tissues⁴. The observations suggest that the change of water properties (in the NMR sense) in the cellular environment is a universal phenomenon which is independent of species and tissue. The interpretation of the

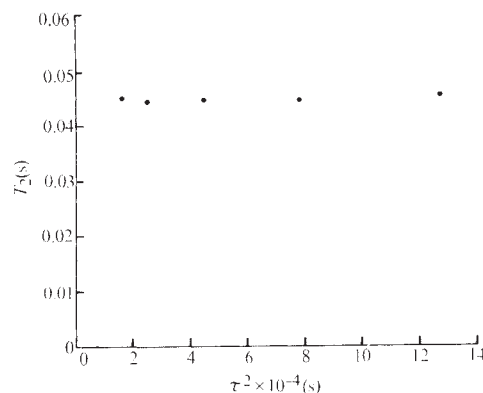


Fig. 1 Spin-spin relaxation time (T_2) of proton in muscle water as a function of observation time (τ).

NMR results, however, is still controversial. Some investigators favour the interpretation that the dramatic shortening of the proton relaxation times implies a structural change of the cellular water; water molecules inside the tissue are more ordered than those of ordinary water^{1,2}, but others do not agree with this interpretation. Hansen and Lawson¹⁰ argued that the high resolution NMR line broadening might be caused by the diffusion of free water through microscopic magnetic field inhomogeneities, although the pulsed NMR results are not consistent with this broadening mechanism. First, the shortened values of T_1 for cellular water cannot be explained by diffusion in an inhomogeneous field. Second, if this mechanism is to be effective in reducing the apparent spin-spin relaxation time, then an unreasonably large value for the local field inhomogeneity must be assumed. It was shown by Carr and Purcell^{6,7} that the measured T_2 in an infinite sample is given by

$$\frac{1}{T_{2(\text{effective})}} = \frac{1}{T_2} + \frac{1}{3} \gamma^2 G^2 \tau^2 D \quad (1)$$

Here, D is the diffusion coefficient, γ is the gyromagnetic ratio, G is the magnetic field gradient and τ is the time between the 90° pulse and the 180° pulse. In a bounded system the effective D is smaller than the true diffusion coefficient¹¹⁻¹³. Suppose we assume an upper limit for D and set it equal to the value for pure water. Then for a typical $\tau \sim 0.03$ s and T_2 measured ~ 0.04 s, a value of $G = 2.2$ gauss/cm would be required, which is more than twenty times the natural sample magnetic field inhomogeneity as determined from the width of the echo signal. Furthermore, we have measured the value of T_2 for muscle water for various values of τ from 13 ms to 36 ms. The values of $1/T_{2(\text{effective})}$ were unchanged within experimental error (Fig. 1). If the relaxation is the result of molecular diffusion through a magnetic field gradient, the reciprocal of the measured T_2 would be a linear function of τ^2 (ref. 10 and equation (1)). This dependence is not observed in our experiments.

Other objections to the ordered water interpretation have also been advanced. For example, Glasel has pointed out¹⁴ that the exchange of solvent species between the bulk phase, average local magnetic field, and that which exists near suspended matter is sufficient to cause line broadening, and no ordering or disordering of the solvent must be postulated. To support this argument, Glasel cites experiments on water in systems packed with glass beads to show that the relaxation times of protons and deuterons change as the surface-to-volume ratio varies. We feel, however, that the results of these experiments do not necessarily negate the ordered water interpretation.

Glass is hydrophilic and has a strong Van der Waals interaction with water, which can be easily seen from the capillary effect. Hori's freezing experiments¹⁵ showed that a thin layer (greater than 1 μm) of water held between two glass surfaces

Table 1 Measurements of Relaxation Times and Diffusion Coefficients

	T_1 (s)	T_2 (s)	$D \times 10^{-5}$ (cm ² /s)
Pure water	3.09 ± 0.15 (4)	1.52 ± 0.093 (9)	2.78 ± 0.035 (11)
Skeletal muscle	0.72 ± 0.05 (6)	0.045 ± 0.002 (12)	1.43 ± 0.07 (5)

All values are mean $\pm$ standard error of the mean. The numbers in parentheses represent the number of samples analysed.

is highly structured. Therefore, it is clear that water molecules must be ordered near the surface of the glass beads used by Glasel¹⁴. As the radius of the glass bead is reduced, the surface area of the glass-water interface, as well as the percentage of structured water, increases. This increase in water order can cause the relaxation times to be reduced. Glasel's experiments therefore do not disprove the ordered water interpretation, but support it. In addition, evidence for the existence of a highly structured water near interfaces is reported elsewhere for physical¹⁵⁻²⁰ and biological systems²¹⁻²³.

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Trickle Perfusion for Organ Preservation

THE two most successful methods of preserving canine kidneys are those described by Collins *et al.*¹ and Belzer *et al.*². In the Collins system, which is extremely simple, the organ is flushed with a solution ('Collins C4') similar to intracellular fluid with a high potassium, phosphate and magnesium content. This solution is left in the kidney stored at 4° C, which will function well when re-implanted after 24 h. It is essential, however, that there should be no warm ischaemic damage to

the kidney³. In Collins's experiments, the animals were very carefully maintained so that the kidneys were not maltreated until they were removed and rapidly cooled.

The Belzer system preserves the kidneys at 10° C for longer periods with better initial function on re-transplantation after 24 h⁴ and is successful with kidneys that have suffered ischaemic damage⁵. The apparatus, however, is complicated, expensive and difficult to transport. Very careful surveillance and continuous perfusion at relatively high flows with a recirculating perfusate containing specially prepared plasma are needed with this method.

Hypothermia is of value in organ preservation. Oxygen consumption of a kidney at 10° C is approximately 5% of normal⁶. Some of the advantages of the Belzer system can be obtained with a much simpler perfusing apparatus using low flows and single passage of perfusate without recirculation⁷. Flow rates of 100 ml./h of a hypertonic dextran containing buffered salt solution at 4° C gave consistently satisfactory preservation of dog kidneys for 18 h and 50% successful preservation for 30 h when the removal of the second of the kidney pair was delayed for 3 weeks. Lactic acid was continuously released and a progressive rise of lactic dehydrogenase in the venous effluent was detected, indicating active anaerobic metabolism but also continued cell damage.

We felt that this approach deserved further study, with kidney and liver preservation. Livers were preserved for 11-12 h, with 3 l. of perfusate passed through the organ, and kidneys for 24-26 h with a perfusion flow of 30-80 ml./h. Preliminary studies with dyes injected into the perfusate showed that at these very low flows, much of the perfusate did not go through the renal cortical capillaries but came out through capsular arterioles directly. We therefore perfused the kidneys with intermittent pulses of more rapid perfusate flow to improve cortical capillary perfusion.

Johnson *et al.*⁸ successfully used plasma protein fractions (PPF)* as a perfusate instead of cryo-precipitated plasma. Our perfusates also contained PPF with additives similar to those used by Belzer and colleagues, or PPF with an equal volume of Collins C4 solution. We have tried to develop a simple perfusing system with better protection of the organ than is possible with unperfused preservation, and eventually to test this system with ischaemically damaged kidneys. It should also be possible to study metabolism of the organ during perfusion, by analysis of the difference between constituents of a perfusate and the effluent, with a view to improving the preservation and developing a better perfusate and perfusion conditions.

Dogs were anaesthetized with 'Nembutal' supplemented with 'Halothane'. Pigs were induced and maintained on 'Halothane' alone. The right kidneys of the dogs were removed without any special preparation; 500-1,000 ml. of 5% dextrose was infused intravenously and the wound was closed. After removal of the kidney, it was flushed with the perfusing solution and this was continued at 4° C. After 24-26 h the kidneys were re-transplanted to the iliac vessels of the original donors⁹ and 500 ml. of dextrose was infused before the opposite kidney was removed. In the liver preservation experiments, the pigs were allowed to breathe spontaneously during 'Halothane' anaesthesia. The liver was removed from the donor and the portal vein and hepatic artery were cannulated and infused with 1 l. of Hartmann's solution at 4° C. The perfusion was continued at this temperature with solution containing PPF. The liver was allografted orthotopically to the recipient pig (technical details have been described elsewhere¹⁰).

Kidneys and livers were placed in sterile polythene bags and the effluent from the renal vein and vena cava collected in the bag and removed by gentle suction. The organs were perfused by a drip with a gravity head of approximately 1 m. The drip line was connected to a simple blood-warming coil placed with

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Table 1 Renal Preservation Experiments

No.	Preservation period (h)	Total volume of perfusion used (ml.)	Perfusate Kidneys	Survival	Comments
1	26	800	PPF+additives *	4 months	Alive. Latest BUN 23
2	25	800	PPF+additives	19 days	Died. Ischaemic renal insufficiency
3	25	1,200	PPF+additives	6 days	Died. Ischaemic renal insufficiency
4	25	1,000	Equal volumes of PPF+Collins C4†	2 months	Alive. Latest BUN 19
5	24	1,000	Equal volumes of PPF+Collins C4	2 months	Alive. Latest BUN 21
6	24	2,000	Equal volumes of PPF+Collins C4	1 month	Alive. Latest BUN 21
7	24	2,000	Equal volumes of PPF+Collins C4	4 days	Died from delayed anastomotic haemorrhage
Livers					
1	11	3,000	Equal volumes of PPF+Collins C4	2 weeks	Died of peritonitis
2	12	3,000	Equal volumes of PPF+Collins C4	2 hours	Acute liver insufficiency

* Additives/l. of perfusate: Penicillin 200,000 U Sodium bicarbonate 20 meq
Hydrocortisone 100 mg Insulin 40 U
Magnesium sulphate 4 meq Mannitol 24 ml. of 10%

† Phenoxybenzamine omitted.

the organ in a 4° C water bath. Oxygen was bubbled through the perfusate before the experiments began. Siphonage of perfusate from a chamber emptying about 20 ml. every 20 min gave the kidney system intermittent perfusion (Fig. 1). In the liver experiments, perfusion was for a shorter time; the ratio of flows to the hepatic artery and portal vein was approximately 1:2 and continuous trickle perfusion was used.

We had difficulty in maintaining steady perfusion, because, if the perfusate was cold when oxygenated, the flow was stopped by bubbles formed in the line, which was exposed to room temperature. Although perfusate was subsequently oxygenated at room temperature, it was difficult to maintain a constant drip rate without intermittent adjustment.

Seven canine kidneys were preserved for 24–26 h using trickle perfusion with solutions containing PPF. After re-implantation and immediate opposite nephrectomy, four dogs are still alive after 1–4 months and have blood urea nitrogen levels of 19–23 mg %. The remaining three animals died in

less than 3 weeks; two from uraemia caused by severe ischaemic renal damage, and one from delayed anastomotic haemorrhage (Table 1). Two porcine livers were allografted orthotopically after preservation for 11–12 h using trickle perfusion with solutions containing PPF. One recipient pig lived for 2 weeks after which it died from peritonitis, and the other died after 2 h from acute liver insufficiency.

In the single passage perfusion system, toxic metabolites and cell debris released into the perfusate are not recirculated, and the perfusate is not depleted of cell nutrients. Filtration, complicated pumps and monitors are, therefore, not necessary. Cell membranes may lose their selectivity in the cold, however, and there may be indiscriminate passage of water and electrolytes into and out of the cell. Leaving a solution resembling intracellular fluid in the vascular tree of the organ is unlikely to cause damage. This is probably why the Collins C4 solution is successful. The perfusate used in the Belzer machine more closely resembles extracellular fluid and, presumably, an organ successfully perfused by this method maintains the integrity of its cell membranes.

The variability of our results could have been due to the difficulties experienced in maintaining flow rates throughout the preservation periods. In these preliminary experiments, a mixture of equal volumes of human PPF and Collins C4 solution is a basis from which to study improved perfusates which will be used in this low flow, single passage organ preservation system. We feel that our preliminary experience is encouraging. We have retained excellent primary function in kidneys after 24–26 h of preservation. In many of our experiments with more complicated perfusion systems, pig liver preservation has been disappointing¹¹. In fact, the longest period of successful preservation of 7 h was obtained by simple flushing with a perfusate similar to that described by Schalm¹² and hypothermic storage without further perfusion. Eleven hours of preservation with trickle perfusion, therefore, represents a considerable improvement.

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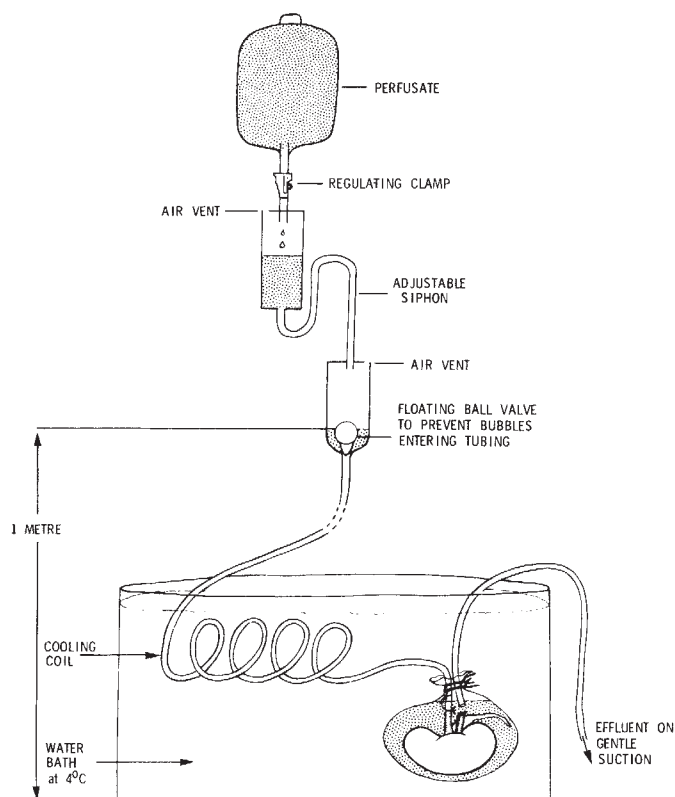


Fig. 1 Low flow intermittent flushing system for hypothermic single passage kidney perfusion.

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Rods Cancel Cones in Flicker

THE retina of man is equipped with two separate receptor systems. Cones, operating best in relatively strong illumination, are the basis of daylight (photopic) vision. In dim illumination (scotopic vision) we rely on rod receptors alone, and the limitations of the rod receptor system are apparent in the character of our visual sensations: in scotopic conditions colour is absent, outlines are blurred and detail is lost. At intermediate (mesopic) levels of illumination, an interesting situation arises: the rod system and the cone system are simultaneously active, and the signals that they generate combine in the production of visual sensations. Unless these rod and cone signals interfere destructively with one another, a mesopic light stimulus must be visible if either rods alone or cones alone could signal its occurrence. Experiments on the visibility of flashes¹ have yielded results consistent with this rule; but in the following experiments with flickering light, the rule breaks down.

An observer directed his gaze at a feeble red spot. His attention was focused on the test patch, a flickering circular field which subtended 3° at his eye and was situated at 5° above his line of sight so that it produced an image on a retinal area well supplied with both rods and cones. The rest was dark except for a steadily illuminated circular background (diameter 8°) which extended over the test patch and was concentric with

it. The background was blue-green (Ilford 603 filter) so that in mesopic conditions it stimulated chiefly rods. The test patch itself was yellow (Ilford 606 filter) so that rods and cones were equally sensitive to it at low intensities. The intensity of the test patch fluctuated sinusoidally with 100% modulation, that is, from twice its average intensity to completely dark. The observer had to adjust the average intensity of the test patch until he could just detect its flicker after prolonged exposure to it.

The results obtained for one observer are presented in Fig. 1. At a flicker frequency of 7.5 Hz (Fig. 1) the results differ considerably from the expected pattern. Instead of becoming more and more conspicuous as the test field intensity increased (vertical scale in Fig. 1), the flicker was visible at scotopic levels of intensity, and again at photopic levels, but not at mesopic levels. Mesopic test patches had clearly visible spatial outlines but looked steady. This mesopic null in flicker was less pronounced at 6.5 Hz (Fig. 1) and absent at 4.5 Hz.

Another unusual phenomenon is apparent in the way that sensitivity to flicker (at 6.5 or 7.5 Hz) varies with background intensity (horizontal scale in Fig. 1). In most visual tests the intensity required for detection of a stimulus against a background increases with increasing background intensity; and at scotopic intensities, the data of Fig. 1 show a typical upward trend. But at higher intensities of the flickering patch the opposite effect occurred: flicker that was not detectable in isolation could be seen when superimposed on a steady background. The dip in the upper set of filled circles in Fig. 1 shows that a background of 1 photopic troland could expose flicker in test patches of from 0.5 to 2 photopic trolands.

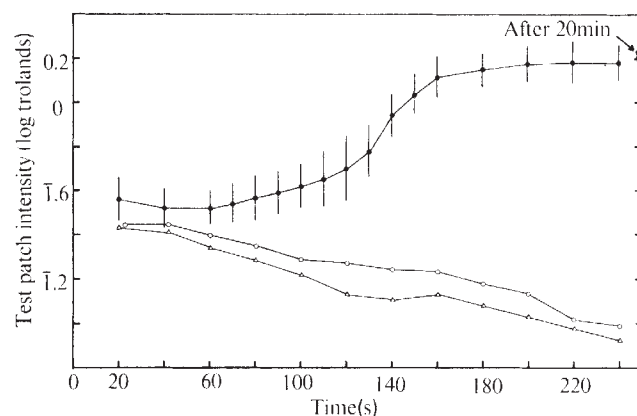


Fig. 2 "Recovery" curves for detection of three rates of flicker after exposure to a blue-green light of 130 trolands which is switched off at $t = 0$. The vertical lines show the range of ten curves for one observer. ●, 7.0 Hz; ○, 4.5 Hz; △, 3.5 Hz.

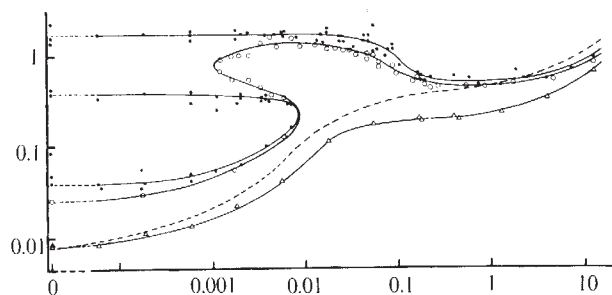


Fig. 1 Flicker detectability contours. The points show the test patch intensities at which three frequencies of flicker are just detectable against blue-green backgrounds of various intensities. At very low test patch intensities flicker is never seen. At high intensities it is visible at all three frequencies. The upper and lower sets of filled circles (7.5 Hz) are separated by a region in which flicker is not seen. The broken line indicates the intensities above which the outline of the 7.5 Hz test patch is visible. Points close together on the horizontal axis represent averages from different sessions. Intensities are in photopic trolands (logarithmic scales). Flicker frequencies: ●, 7.5 Hz; ○, 6.5 Hz; △, 3.5 Hz.

This unmasking of mesopic flicker by a background seems to depend on rod activity: it never occurred in foveal observation where rod involvement is slight. Some other evidence that indicates a dependence of unmasking on rod function is presented in Fig. 2. There the background was more intense than any used in the experiment of Fig. 1, but it was exposed before, and not during, the measurements. The curves represent the time course of flicker sensitivity after the light has been switched off. The observer continuously adjusted the intensity of the test patch, increasing it when flicker was not visible and decreasing it when flicker could be seen. The curve for 7 Hz shows a decrease in sensitivity in the dark. Like the recovery of sensitivity found at lower frequencies, this loss of sensitivity shows the slow time course that is characteristic of the return of sensitivity in rods. Therefore the abolition of flicker at 7 Hz is probably a consequence of rod intrusion; some kind of destructive interference between rods and cones is indicated.

Destructive interference might originate in either of two ways: by cancellation or by inhibition. Non-recurrent inhibitory

connexions linking the rod system to the cone system might, if they exist, allow each flicker signal to destroy the other. The cancellation explanation is simpler. Rod and cone signals might be conveyed without any interaction to a nerve centre that simply adds them together—destructive interference could occur as part of this summation process, if there were a phase difference between the signals. The likelihood of such a phase difference is high, since various experiments²⁻⁵ have shown that the rod response occurs a little later than the cone response. If it is to account for the anomalies of mesopic flicker sensitivity, the phase difference must amount to about 180° at frequencies that show a mesopic null in flicker. Then peaks in the cone signal will coincide with minima in the rod signal and, if the two signals are approximately linear records (with equal amplitude) of the sinusoidal intensity fluctuation that elicited them, they will cancel when combined. A steady signal will result.

If this interpretation is correct it must be possible to eliminate the mesopic null by delivering stimuli asynchronously to rods and to cones. By compensating here for the difference between the phase lags of the two receptive systems, the signals generated by rods and by cones can be brought into phase with one another. Being in phase, they cannot cancel, and flicker should appear.

This revival of flicker by phase adjustments has been successfully achieved, using a blue-green light to stimulate rods (Ilford 603 filter, average intensity = 0.014 photopic trolands) and a deep red light (Ilford 609 filter, 1.5 photopic trolands) to stimulate cones. The two beams of light were both confined to the test patch. At 7 Hz and 100% modulation, either of these stimuli by itself flickered unmistakably, but if both together were admitted to the eye the flicker disappeared. Yet if a phase difference of 180° (a time interval of 72 ms) was introduced between the two, so that the green light was at its brightest when the red light was at its dimmest, the flicker became conspicuous. For one observer, the flicker became noticeable if the green beam was advanced in phase by more than 60°, or retarded by more than 40°.

Each beam formed a compact image in the plane of the pupil. Because cones, but not rods, are differentially sensitive to light incident on the retina from different directions¹, this allowed a direct test of the assumption that the red beam worked mainly through cones and the green beam through rods. The red beam intensity that gave least flicker (when in phase with the green beam) was determined in three conditions: first with both beams passing through the centre of the pupil, and then with either the green beam or the red beam displaced by 3.5 mm toward the temporal margin of the pupil. The cancelling intensity was not measurably different for the two points of entry of the green beam; the green beam therefore worked through rods. But when the red beam entered at the margin, its intensity had to be increased four times in order to abolish the flicker. So the red beam worked through cones.

Paradoxically then, flicker is invisible only when the light stimulus for rods is approximately in phase with the light stimulus for cones. This supports the cancellation hypothesis, for it is only when the stimuli are in phase that the postulated phase shift yields signals that can cancel. By creating that phase shift, the slowness of rods makes it possible to account for the mesopic null while retaining the assumption that the signal passing from eye to brain is simply the sum of the signals generated by rods and by cones.

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Piezoelectric Activity in Invertebrate Exoskeletons

PIEZOELECTRIC effects have been demonstrated in several biological structural materials, such as wood and bone¹⁻³, and in various organic polymers⁴. Additionally, piezoelectric activity has been detected in the otoliths of some species of bony fishes⁵, where it is believed to be involved in sensory perception. On the basis of these findings it seemed worthwhile to assay the integuments of various invertebrates for piezoelectric activity, for they are related in structure, function, or composition to the materials listed above. Accordingly, a number of mollusc shells and carapaces of several arthropods were tested for evidence of piezoelectric properties.

Table 1 Impact Response of Mollusc Shells and Arthropod Carapaces to 5 g Weight dropped 10 cm

Organism	Electrical activity (peak-to-peak amplitude, mV)
(1) <i>Tellina angulosa</i>	50-65
(2) <i>Tellina alternata</i>	50-60
(3) <i>Arca zebra</i>	10-15
(4) <i>Anadara transversa</i>	20-30
(5) <i>Spisula solidissima</i>	35-40
(6) <i>Heterodonax bimaculatus</i>	4-6
(7) <i>Anomia aculeata</i>	12-15
(8) <i>Pecten gibbus</i>	8-12
(9) <i>Ensis directus</i>	50-80
(10) <i>Limulus polyphemus</i>	150-250
(11) <i>Callinectes sapidus</i>	30-40
(12) <i>Homarus americanus</i>	15-25

All readings into a 100 kohm load, ten readings per specimen.

Initial attempts to detect and measure piezoelectric activity by conventional sweep techniques, using thin, flat ground, polished specimens, produced ambiguous results; resonance and anti-resonance peaks of small amplitude were noted at many frequencies within a sweep range of 30 kHz to 2.5 MHz, reflecting the inhomogeneous structure of the material. To permit rapid, non-destructive, and unambiguous testing of the samples an impact technique was finally chosen and carried out as follows.

Samples were rinsed several times in distilled water and allowed to air-dry. Opposing electrodes were then painted on each surface with conductive silver paint (Dupont No. 4916). After the paint had dried overnight at room temperature, thin copper wire leads were attached to the electrodes with a small dab of low-melting point solder ('Cerroseal 35', melting point 110° C). The leads were then connected to the vertical amplifier input of a high-sensitivity oscilloscope, and recordings were made of the impact response to a 5 g lead weight dropped from a height of 10 cm onto the sample. Peak-to-peak amplitude of the initial electrical impulse was noted, and is presented in Table 1 for all samples tested. In all cases electrode area was 5 cm² ± 0.5. Care was exercised when setting up the equipment to ensure that all connectors, cables, and measuring apparatus were non-microphonic. The oscilloscope and test area were acoustically isolated, and the connecting coaxial cable was wrapped in foam rubber. The leads from the sample under test were soldered directly to the ends of the coaxial cable.

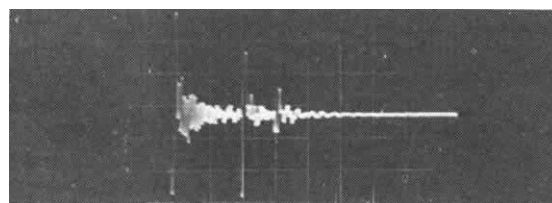


Fig. 1 Electrical response of *Tellina angulosa* shell to 5-g, 10-cm impact. Load resistance 100 kohm. Horizontal scale, 1 ms division⁻¹; vertical scale, 10 mV division⁻¹.

¹ Stiles, W. S., *Proc. Roy. Soc., B*, **127**, 64 (1939).

² McDougall, W., *Brit. J. Psychol.*, **1**, 78 (1904).

³ Arden, G. B., and Weale, R. A., *Proc. Roy. Soc., B*, **142**, 253 (1954).

⁴ Gouras, P., and Gunkel, R. D., *Doc. Ophthal.*, **18**, 137 (1964).

⁵ Gouras, P., and Link, K., *J. Physiol.*, **184**, 499 (1966).

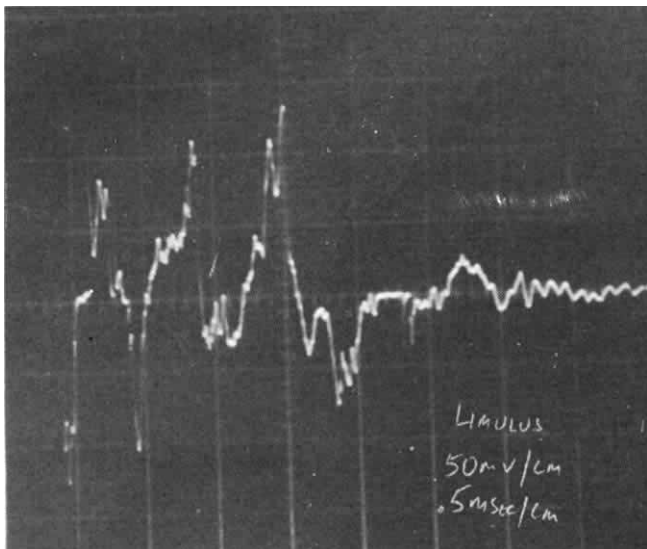


Fig. 2 Electrical response of *Limulus* carapace to 5-g, 10-cm impact. Load resistance 100 kohm. Horizontal scale, 0.5 ms division⁻¹; vertical scale, 50 mV division⁻¹.

Photographs of typical responses from what might be termed an active mollusc shell (*Tellina angulosa*) and an active arthropod carapace (*Limulus polyphemus*) are presented in Figs. 1 and 2. In air each shell is characterized by a typical signature in response to impact, determined principally by its piezoelectric activity, its geometry, electrode location, and sound velocity and absorption within the shell material; standing waves generated by the initial impact bounce back and forth within the structure, generating a voltage whenever they impinge on the electrodes. If the shell is placed in a damping medium, such as water, only the initial response is observed. Fig. 3 represents the signal recorded from a *Tellina angulosa* shell half immersed in a beaker of water, with the electrode area above the water line, when a 10 g lead weight is dropped from a height of 10 cm into the beaker.

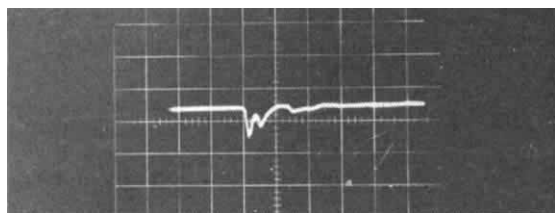


Fig. 3 Electrical response of *Tellina angulosa* shell in water. Horizontal scale, 2 ms division⁻¹; vertical scale, 1 mV division⁻¹.

It is clear that many mollusc shells, and some arthropod carapaces as well, exhibit piezoelectric activity to a varying degree, resulting in some cases in the generation of voltages of sufficient magnitude in response to mild mechanical shock to have possible biological significance. It would be tempting to infer that this activity serves a sensory function, especially in those molluscs, such as the razorback clam, which seem able to perceive sound, despite the apparent absence of recognizable sense organs.

It is also interesting that the mechanism of shell formation described by Digby⁶—involving material deposition under the influence of a steady polarizing electric field generated by the periostracum—is very similar to the process used in industry to polarize piezoelectric ceramics and other materials^{7,8}, and would suggest that piezoelectric properties would be found in the calcified shell.

I thank Professor K. J. Boss for specimens and for helpful

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- ¹ Fukuda, E., and Yasuda, I., *J. Phys. Soc. Jap.*, **12**, 1158 (1957).
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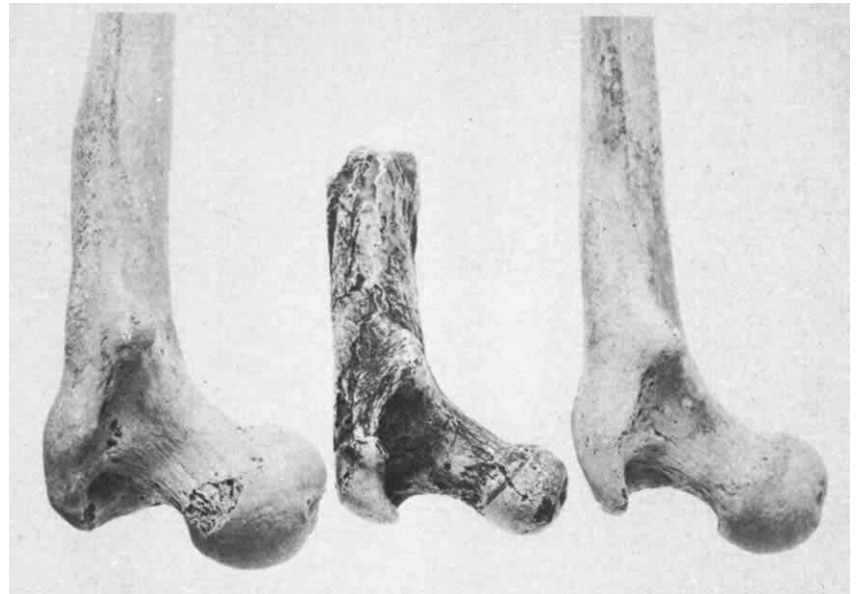
Proximal Femoral Anatomy of *Australopithecus*

BECAUSE of the importance of the femur in the gait of bipeds, it is surprising that only moderate attention has been paid to the samples of proximal femora of *Australopithecus* for which published descriptions and/or casts are now available (STS-14, SK-82, SK-97 and OH-20). In descriptions of these specimens, several morphological features have been claimed to separate them significantly from later Pleistocene hominids¹⁻⁴. We consider that only two features may lie outside the normal range of variation of *Homo sapiens*.

In the original description of the STS-14 fragment, Broom and Robinson reported¹ that the inter-trochanteric "crest" was "not well developed", and Day has reported it to be absent in the Olduvai specimen (OH-20)² and suggested that this indicates "that the capsular ligament of the hip joint had no thickening of its ilio-femoral portion". Our observations of modern specimens show that the development of the inter-trochanteric line is not necessarily an indicator of the mass of Bigelow's ligament or its tension during life. This is shown by the occasional absence or minimal expression laterally of the trait in specimens which also display clearly marked medial synovial reflexions (an area where the capsule and capsular ligaments are of necessity slackened to allow hip abduction). Furthermore, the inter-trochanteric line is usually not present in adolescents and its development to some extent is therefore related to age. Even in adults, the expression of this trait is highly variable; it ranges from almost complete absence to a marked, elevated rugosity. To quantify this variation, we surveyed ninety-two femora of an Amerindian skeletal population. The degrees of expression and their relative frequencies were classified as follows: (1) Complete absence (0.18)—no variation in cortical topography in the region where the trait is normally expressed (although colour variation often indicates the extent of Sharpy's fibres along the ligament's insertion); (2) trace to slight (0.33)—presence of a faint to easily recognizable line as a result of surface variation in the cortex; (3) moderate (0.30)—presence of a low rugosity; (4) pronounced (0.18)—presence of a marked rugosity.

Although the Olduvai specimen may lack cortical variation in the region of the inter-trochanteric line, Broom and Robinson's description¹ indicates that there was at least a slight expression of the trait in the STS-14 fragment and, furthermore, the SK-82 and SK-97 proximal femoral fragments do display the trait, including an easily recognizable medial synovial reflexion. To be classified as before these specimens would have to be placed in category (2), the most frequent type in the surveyed population. Although the subdivision of any continuous variation is artificial, it is clear that at least 51% of the human femora studied showed equal or less development of the inter-trochanteric line than do three of the four australopithecine specimens.

Fig. 1 Posterior view of two modern femurs (KSU-043 and KSU-905) and SK-97 (cast). The femoral necks have been placed horizontal to eliminate anteversion. The distance between the shaft and the lesser trochanter's most medial extension is (from left to right): -1 mm; 0 mm; and -6 mm.



Broom and Robinson noted that although the lesser trochanter on the STS-14 fragment had been broken off, it could be readily restored and appeared to be more laterally placed than in modern man¹. Day², Napier³ and Le Gros Clark⁴ reached similar conclusions with respect to the Olduvai and Swartkrans femoral fragments.

Consideration of only the static position of the lesser trochanter on the femoral shaft can lead to erroneous conclusions. Anteversion of the femoral neck is highly variable in *Homo sapiens*, both within and between different populations, and as the femur becomes increasingly anteverted, the position of the lesser trochanter will become progressively more medial in functional anatomical position. Assertions that the position of the lesser trochanter in *Australopithecus* differs significantly from that of modern man are not substantiated if attention is paid to the range of variation of *Homo sapiens*. To illustrate this, the Amerindian population was again surveyed with regard to the position of the lesser trochanter. All specimens were viewed posteriorly with the femoral neck horizontal in order to eliminate anteversion (see Fig. 1). The distance from the most medial extension of the lesser trochanter to the medial edge of the femoral shaft was measured for each specimen. Where the trochanter failed to reach the edge of the shaft, the measurement was considered negative, and where the trochanter ex-

ceeded the edge of the shaft by its medial extension, the distance was recorded as positive. The results were found to be normally distributed and a normal curve could therefore be fitted to the data (Fig. 2). Identical observations were made on casts of the SK-82 and SK-97 specimens. It may be noted that both australopithecine specimens lie well within one standard deviation of the mean of this modern population.

Day has suggested² that the greater trochanters of the Olduvai and Swartkrans specimens are relatively smaller than those of modern man. Our observations do not support this conclusion. First, the more laterally placed shaft in the australopithecines (our unpublished work) causes the trochanter to be assimilated more into the proximal mass of the femur, thus giving the illusion of smaller size. Second, although the size of the trochanter is difficult to assess metrically, approximation of its area can be obtained by simple measurements of its maximum height and breadth. The SK-97 femur is not large, and trochanteric measurements were therefore made on twenty-five modern femurs of 40 to 45 cm in maximum length. These gave means of 31.4 for width ($\sigma=2.79$) and 35.6 for height ($\sigma=2.30$). The same measurements made on SK-97 (which, in spite of slight reconstruction, has the most intact trochanter) are 32 mm for height and 32 mm for width.

Only the small femoral head and long femoral neck of *Australopithecus* are thus outside the human range. By themselves, these features cannot be taken to indicate functional differences in mode of gait in this hominid without normalization by length and more formal biomechanical analysis of the pelvic-femoral complex.

Note added in proof. Since the submission of this article, Dr Phillip Tobias has kindly provided an additional survey of inter-trochanteric line development in one hundred Zulu femora. Utilizing the categories defined above, he obtained the following results: (1) 0.05; (2) 0.27; (3) 0.43; (4) 0.25.

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³ Napier, J. R., *Arch. Biol., Liège*, 75, Suppl. 673 (1964).

⁴ Le Gros Clark, W. E., *Man-Apes or Ape-Men* (Holt, Rinehart, and Winston, New York, 1967).

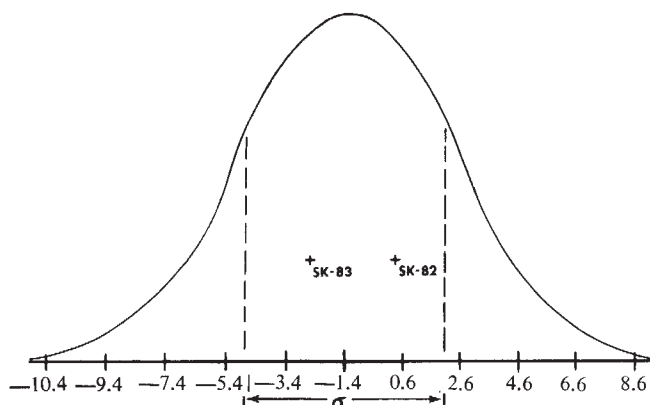


Fig. 2 Normal curve fitted to sample of ninety-six human femora (ninety-six individuals) measured for the position of the lesser trochanter with respect to the femoral shaft (all measurements in mm; compare Fig. 1). Identical measurements made on the australopithecine specimens (casts) are -3 (SK-82) and 0 (SK-97). The mean of the population was -1.4 mm ($\sigma=3.6$; range = +8 to -8.5; chi square for fit of data to normal distribution was $P=0.26$).

BOOK REVIEWS

Birds in the Field

Ecological Isolation of Birds. By David Lack. Pp. xi+404. (Blackwell Scientific: Oxford and Edinburgh, 1971.) £4.25.

THIS book sets out to provide detailed evidence for the hypothesis, formulated by the author 25 years ago, that ecological isolation exists between bird species in nature. At the time, the view was hardly accepted, the chief criticism being that many closely related species were known to share the same habitat and were thought to have similar ecology. Recent research, however, has established "competitive exclusion" as a fundamental biological principle, and food has been shown to be the limiting factor in the widest variety of cases.

Dr Lack's introduction includes a particularly interesting historical review. The next twelve chapters examine ecological isolation in a number of related, and usually sympatric, forms (especially titmice and finches) or the avifauna of circumscribed areas (especially islands). A final chapter summarizes the important points that have emerged and considers such problems as the acquisition of segregation by feeding, and the consequences for species' origins and diversity. A quarter of the book consists of appendices tabulating in fuller detail the ranges, habitats, feeding stations and food of bird groups that have been subjected to field research.

One value of the text lies in having so much material synthesized in readable form. It is, indeed, extremely readable and is much enhanced by Robert Gillmor's delightful drawings. The gaps in our knowledge are now more evident. It is obvious that certain avian groups require further study and that new approaches—such as the mathematical considerations of MacArthur—may be helpful. The trends, usually referred to as Bergmann's and Allen's rules, of increasing body size and smaller appendages in colder regions are so widespread that they are firmly accepted as adaptive, although, as Dr Lack points out, it is not clear precisely what they are adaptations for. The trends were formulated for races of the same warm-blooded species but they seem to apply also within genera. Dr Lack suggests that it may be doubted whether climatic factors ever limit the ranges of birds directly. In support, it could be said that many tropical species are, with adequate food supplies, kept healthy in

temperate "wildlife parks". Intraspecific differences become apparent, however; tropical water birds with long legs and broad feet commonly suffer frostbite in severe weather and lose their toes, a situation which might well impair feeding efficiency in the wild. Koskimies also showed differences between species of day-old ducklings in their ability to maintain normal body temperature in cold surroundings, and inferred that climate placed limits on their typical ranges. Further investigation along these lines seems to be required.

The influence of Dr Lack's work on a generation of ornithological research has been enormous. His latest book is a timely and succinct examination of field studies largely generated by his own earlier ideas. JANET KEAR

Thermodynamic History

From Watt to Clausius: The Rise of Thermodynamics in the Early Industrial Age. By D. S. L. Cardwell. Pp. xv+336. (Heinemann: London, October 1971.) £5.00.

DONALD CARDWELL describes his book as follows: "This, then, is an account of a scientific revolution (thermodynamics) that took place between about 1790 and 1865, one of whose root causes was the rapid progress made in power technology during this period. It is not a general history of theories of heat". Unfortunately, the focus on thermodynamics is blurred by the intrusion of too much general history.

Thus, the early chapters are confusing fragmented compilations of ideas, observations and experiments regarding the diverse phenomena of heat. Cardwell readily admits the confusion but attributes it to the failure of contemporary scientists to see the emerging new cosmology of the universe as a cosmic heat machine, a view supposedly only attained in the 19th century. He has therefore included discussions of meteorology, radiation and conduction even though he never shows in what way thermodynamics brought unity to this diverse collection. His treatment of the culmination of thermodynamics with Kelvin and Clausius, for example, includes no significant analysis of any of these phenomena, and, in order to consider the winds in relation to thermodynamics, a leap well beyond 1865 to Napier Shaw's *Manual of Meteorology* must be made. The scientific revolution in thermodynamics arose within a far more restricted province in

the theory of heat, namely, the fruitful interaction between steam power technology and scientific interest in the thermal properties of gases, vapours and liquids. It would therefore have been far more appropriate to discuss Lavoisier's caloric model of a liquid and its vapour than to review Rumford's work on conduction and convection.

When Cardwell does turn to the interaction between technological reality and scientific ideas, we are treated to a brilliant analysis of how water power technology contributed to Carnot's stunning achievement. His use of the waterfall analogy for the temperature fall of caloric from boiler to condenser, for example, an image made possible only with Watt's engine and the separate condenser, seems a quite natural step when viewed with high pressure water piston engines operating so similarly to their steaming counterparts in the background. Moreover, Carnot's introduction of a reversed steam engine functioning as a refrigerator is shown to have a corresponding conceptual precedent in 18th century thought experiments which reversed waterwheels to perform as pumps. Thus, Cardwell demonstrates that there was a natural flow of ideas from a realm of technology that was well understood, water power, to one that represented a persistent puzzle, namely, the overwhelming superiority of high pressure over low pressure steam engines. Carnot's unique insight that it is the higher temperature rather than the higher pressure which really rules steam power is seen as a watershed in the science of thermodynamics.

Following his illuminating discussion of Carnot's thought, however, Cardwell becomes more routine in his outlining of the contents of major thermodynamic papers by Clapeyron, Clausius and Kelvin. Rankine, although mentioned in a chapter title, is dismissed as somewhat incomprehensible and as one who addressed himself primarily to engineers. But if engineers managed to understand him (and they did), he could not have been all that incomprehensible; and if thermodynamics resulted from the interaction of science and technology, why should the one thermodynamicist who made a concerted effort to cover the whole gamut of heat behaviour, from a vortex atom that could account for radiation to detailed analysis of engine behaviour, from the graphical portrayal of thermodynamic concepts to the highly abstract symbolism of energetics, be excluded?

Donald Cardwell has a genius for dismembering persistent myths in the history of science. For example, he deals incisively with the recurring tale that Watt was supposedly indebted to Black's discovery of latent heat before he paid attention to the waste of steam and the consequent loss of power in the Newcomen engine due to the repeated cooling of the cylinder with injected condensing water. Were that really true, Cardwell observes, then we would have a veritable paradox since the high latent heat which Black had discovered should have appeared to be a positive advantage in reducing the amount of condensation and thus helping to maintain steam supply. I hope that Cardwell will press on to further demythologizing in the history of thermodynamics and lead us beyond the standard fare of just Clausius and Kelvin.

EDWARD E. DAUB

Man and Machines

IEE Centenary Lectures. 1871-1971—Electrical Science and Engineering in the Service of Man. Pp. viii+183. (The Institution of Electrical Engineers: London, 1971.) £5.00.

OFFICIALLY this is a record of lectures delivered at the Centenary celebrations of the Institution of Electrical Engineers. Its value is certainly much higher. Research scientists probing deeper and deeper into their special subject on a narrower and narrower front become less aware of the activities of their fellow scientists and are less able to read the learned papers of their colleagues. This volume provides an opportunity to read lectures by experts which are written at the level of the non-specialist.

It is not merely an acknowledgment of a century of progress—a milestone in electrical engineering. It is a book to be read and enjoyed by all who dare to think. It takes the whole universe as its setting. Nor is it enough to say that scientists may read for pleasure of the activities of their colleagues. At this time it is almost a duty to do so, for I believe that progress now comes fastest when cross-pollination takes place. It is important that an expert in electrical machines should learn first hand from an authority such as Professor Hodgkin of the nature of nerve impulses. The expert in another discipline may be the person required to fit in that most important piece of the jigsaw which the other authority lacks. There is no doubt that all the authors could have said much more on their subject, given the time and space to do so, and this is perhaps one of the few regrets one may feel about the book as a whole.

The title *IEE Centenary Lectures* is, I fear, not likely to attract many readers outside the profession, and in particular young people, for whom it would surely

serve as the counter to all those who predict the fall of mankind through science. The subjects of the eight lectures which form the main text are seen to be chosen in this context; for example, "Energy, progress and man" and "Disparate world: challenge to education" are two of the titles. Sir Brian Flowers's opening address sets the tone in his last sentence: "He (the engineer) will understand that, in AD 2000, we shall need primroses as much as power stations".

The approach of all the authors is positive—no mere blaming of the scientist as the instrument of doom. Nor is the text "heavy" reading. The opening lecturer felt he had a choice between writing something which was learned but dull or entertaining but silly. I am certain that he succeeded in combining the best of both, as did the other authors, and produced material which was both learned and entertaining. With a title like: "A journey through science from 1871 to 2000", this book might have appeared in all school libraries. I feel it a duty to encourage this. The institution itself might, even at this stage, introduce an exciting dust cover, ensure advertisement of it in such publications as *School Science Review*, and recommend it for sixth form reading wherever possible.

E. R. LAITHWAITE

Using Radioisotopes

Radioisotopes in Medical Diagnosis. By E. H. Belcher and H. Vetter. Pp. x+800. (Butterworth: London, August 1971.) £19.00.

THE original book by Veall and Vetter on *Radioisotope Techniques in Clinical Research and Diagnosis* appeared in 1958. Those of us for whom the book has long been a standby have been keenly awaiting a new edition.

The long expected volume has now appeared, albeit with one of the leading names changed and in a multi-author format. With the advances in techniques it was inevitable that the new book should be larger than the old.

In the first chapter, Veall discusses radioisotopes and the various radiations emitted. The section of the chapter dealing with labelled compounds provides a good example of the drawback of the multi-author approach. The Wilzbach technique is described on page 17 and again on page 232 in the chapter by Pearson. The authors of these two chapters are unable to decide whether we have isotope "cows" or cows and indeed whether we "milk" or milk them.

The second chapter contains a good account of the production of the scintillation spectrum and of the various radiation detectors. The next chapter describes the electronic circuitry used in counting equipment. It ends with a

section on fault tracing which is much too short to be of practical use and is surely out of place in a book of this kind.

The fifth chapter is a contribution by Belcher, one of the editors, on *in vitro* counting methods. Here again there is some overlap with a previous chapter.

Trott's chapter on *in vivo* measurement gives a very good account of linear scanning. There is an unfortunate error in the heading of Table 6.3—"principle" for "principal". The chapter contains illustrations of brain scans. There are similar illustrations in Mallard's chapter some 500 pages later. This highlights one of the defects of the book—almost all the chapters are individually excellent but there is too little cohesion between them.

Chapters on radiation dosimetry and safety aspects follow, and the first part of the book ends with a section on compartmental analysis by Matthews. For readers who are unused to grappling with mathematical symbols, a few practical examples, fully worked out, would have helped enormously.

Most of the remaining chapters are devoted to the investigating of various body systems using particular isotopes. Haxhe explains the principle of dilution analysis in the estimation of body water, extracellular fluid and so on. The simultaneous measurement of these is described in detail—but does anyone indulge in these heroics nowadays? The limited value of such determinations is now recognized and it is perhaps significant that almost none of the 130 references is less than eight years old. One strange point is that body weight is consistently and wrongly written as one word.

It is disappointing to find a chapter on calcium studies which makes no reference to the work from King's College Hospital on the "random walk" concept.

Although all these chapters are authoritative, I feel most of them could have been considerably shortened without undue loss.

Towards the end there are interesting chapters on methods of studying steroid metabolism by Cope and on *in vitro* tracer tests by Ekins. In the final chapter, Tubiana is given the impossible task of trying to describe the therapeutic uses of radioisotopes in seventeen pages. The section is interesting but is not relevant to the rest of the book and it is a pity that it was included.

Clearly this is a book which should be in every medical radioisotope laboratory. It could by severe editing be made considerably shorter. I also have the feeling that the book could have been written five years ago. Indeed, judging by a footnote, some of it was. As to the price, "outrageous" is scarcely adequate.

J. P. NICHOLSON

CORRESPONDENCE

Blueprint for Survival

SIR,—“Blueprint for Survival”¹ attempts, at some length, to set out the facts of the present world situation with regard to population, resources and environment. It then goes on to make a number of predictions based on current trends and socio-political attitudes. Its signatories, although they may not agree with every point of detail, are convinced that the assessment it contains is substantially correct. We also believe that its suggestions for reversing some of the more obviously damaging trends are sensible.

You accuse *The Ecologist* and the signatories of reflecting many of the “half-baked anxieties about what is called the environmental crisis” and go on to suggest that “the forces which have made civilized societies more humane” will overcome our problems (*Nature*, **235**, 63; 1972).

It is surely your duty, both to the scientific community and to the society at large, to marshal and present *in extenso* the evidence which leads you to dismiss the arguments in “Blueprint for Survival”.

Yours faithfully,

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¹ *The Ecologist*, **2**, 1 (1972).

SIR,—The leading article in *Nature* entitled “The Case against Hysteria” (*Nature*, **235**, 63; 1972) does little credit to a distinguished journal, from which one expects impartial and well balanced assessments of scientific and social problems. Those who are criticized for the views they advance may have overstated their case at times, but such overstatements are the result of their awareness of the urgency of the problem that faces mankind (not just Britain) if population growth is not checked in the next few decades. You, Sir, appear to think that the problem will cease to exist if you bury your head in the sand. A more constructive discussion of these problems in your columns would be very welcome.

Yours faithfully,

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Improving the Turkey

SIR,—The leading article in the Christmas *Nature* (**234**, 429; 1971) focused the essential problem raised by the Rothschild Report on to the timely question: “Is it feasible to think of developing Christmas turkeys weighing substantially more than those on the market and, if so, what kind of research programme would be necessary and how much would it cost?” Now members of the staff of my laboratory did almost exactly that a few years ago. Not quite exactly, because the problem is not so much to increase total weight until the bird is too big for modern ovens or less philoprogenitive families, but to get it to a suitable weight with as little input of food as possible, and with its weight concentrated in the most desirable part, the breast (over-portly males find difficulty in copulating, and the only way round this difficulty lay in the development of satisfactory methods of artificial insemination). The story of how the research was actually organized is perhaps worth telling because it provides a concrete example of the inadequacy of either the Rothschild or Dainton proposals.

One and perhaps the most important factor in the recent “industrialization” of large scale production of poultry, including turkeys, has been development of systems of breeding and testing lines which will be crossed to produce the actual birds which will be sold as carcasses or egg layers. These systems are based on general genetic principles, but the efficiency of a firm depends critically on how well it can squeeze the last few per cent out of the output/food input ratio. In the early days of the industrialization, the situation was highly competitive; this phase has now been replaced by one of worldwide mergers. In the mid-fifties, a turkey breeder asked the ARC to advise him on his genetics. I have forgotten whether it was the then Secretary (Sir William Slater) or the then President (Lord Rothschild) who asked me to get one of the people in my ARC Unit to look into the question; but at any rate the Research Council set-up worked satisfactorily as an “indirect customer” at the first stage. But it soon became clear that we could not get the detailed information necessary without entering into a relation of confidentiality. When the ARC were consulted, they regretfully came to the conclusion that they could not allow one of their staff to advise one turkey breeder out of the many on a basis of confidentiality, since they would not have enough trained staff if his competitors put their claim for similar official assistance. Even less

could the ministry become closely enough associated with the details of the production process in a particular firm. The kind of operation which the ministry could undertake is exemplified by its Poultry Stock Improvement Plan, which involved building four large strain-testing stations in various parts of the country at a cost of at least £2 million; when the scheme turned out to be a disastrous failure (owing to lack of proper genetical advice) this investment had to be, to all intents and purposes, written off.

The only solution, if we were not to drop the turkey project entirely—and it offered too many intellectually interesting problems for that to be acceptable, even if we paid no attention to the economics—was to arrange a swap of posts between the university departmental staff and the staff of the ARC Unit. The turkey geneticist took a university lectureship, at considerably reduced salary, but with the normal permission to engage in paid consultant work to a limit which was deemed not to interfere with his duties as a university teacher. A few years later this turkey firm became the centre of a large amalgamation and now forms the nucleus of one of the largest turkey breeding organizations in the world*; and the turkey geneticist was asked by the Ministry to write their official handbook on turkey breeding. The total cost to the country was one lecturer-level salary (which would have had to be paid anyway, even if the man had not worked on turkeys).

So all ended on a suitably Christmassy note. But there are two morals in connexion with the Green Paper. The first is that the real “customer” is usually the actual producer, and not the ministry as Rothschild suggests; in fact neither the ministry nor even the less encumbered research council can represent the customer in the full detail which may be necessary. The second is that even the ultimate customer often does not know at all concretely what it is that he is after—the turkey breeder only knew that he wanted to improve his stock, not that he had to set up an elaborate system of line breeding, crossing and testing. To tell him that, he needed a “contractor” who could enter into a confidential professional relationship.

Yours faithfully,

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* They can produce big turkeys, too. I think they still hold the world record with something over 65 lb. If the Editor of *Nature* wants their address before next Christmas, I shall be happy to supply it.

Obituary

Professor Sir Ronald Nyholm



Photo by Godfrey Argent

THE tragic death of Sir Ronald Nyholm in a car accident on December 4, 1971, will come as a sad blow to all who have ever come in contact with him.

Born in 1917, Ronald Nyholm graduated from the University of Sydney, Australia, in 1938, and then joined the staff of Sydney Technical College before moving to England as an ICI Fellow in 1947. Nyholm was one of the immediate post-war group who founded in University College Chemistry Department an active colony of Australian chemistry which persists to this day. At University College he readily interacted with the local attitude to chemistry and found, in particular, rapport with the philosophy of Sir Christopher Ingold who was the head of the department. After returning to Australia in 1952 as an Associate Professor of Chemistry at the University of Technology in Sydney he assumed a chair of chemistry at University College London in 1955, where he became head of the department in 1966 on the death of Professor E. D. Hughes.

Ron Nyholm's impact on inorganic chemistry has been tremendous. He was one of a small band of inorganic chemists who in the immediate post-war years established the present discipline and transposed it from the essential factual approach of the pre-war years to the physico-chemical based discipline of today. In particular his interest in transition metal chemistry, coupled with his fluency in lecturing and presenting an attractive thesis, helped to direct the course of inorganic chemistry in Britain.

The title of his inaugural address at University College, *The Renaissance of Inorganic Chemistry*, was apt; in this, he developed the theme that was to prove his basic philosophy for the next sixteen years, namely a total integration of chemistry, with physical chemistry providing a tool for the rationalization of inorganic chemistry and with organometallic chemistry as a natural and fruitful bridge between inorganic and organic chemistry.

Nyholm's initial research was concerned with the coordination chemistry of the later transition elements with monodentate arsine ligands, an area very much in the tradition of Australian inorganic chemistry as initiated by G. J. Burrows. Although Nyholm never published a paper with Burrows he often recounted his debt to this worker who in many ways he considered his mentor in this field. Much of the early work was carried out in collaboration with that other great Australian coordination chemist, Frank Dwyer, and it is of interest that together these workers have spread their influence over much of Australian and English inorganic chemistry.

On his arrival at University College, Nyholm took the opportunity to review the factors that influenced the stereochemistry of the group VIII elements¹ and became interested in the potential use of magnetic measurements in elucidating stereochemical problems for the transition elements. This is a theme that he proceeded to develop with considerable success for the next twenty years. His application of magnetic measurements to chemical problems was the first of a series of adaptations of physical methods to inorganic chemistry and his interest in the development of instrumentation was an important factor in his work. He had the facility to recognize the potential use of many of the newer physical methods that developed after 1950, and was a prime mover in the adaptation of vibration, electronic and magnetic spectroscopy in the widest possible forms to inorganic problems. Nyholm was nevertheless primarily a synthetic inorganic chemist who always enjoyed preparing a new compound.

The initial preparative work he carried out in London was in the area of polydentate arsine derivatives, and after some work on tertiary arsine complexes, his attention was taken with the di-tertiary arsine, orthophenylenebisdimethylarsine (diarsin), initially discovered by

Chatt and Mann. This ligand provided Nyholm with the degree of versatility he was seeking and for the next twenty years he proceeded to use this as a stabilizing group to uncover larger areas of interesting transition metal chemistry. His paper on the trivalent and tetravalent complexes of nickel² was the first of a series of publications dealing with uncommon oxidation states of transition metal ions stabilized by diarsine. He further utilized this ligand in establishing the then unusual coordination number, and stereochemical arrangements of three, five, seven and eight groups for a variety of elements and in particular the arrangements of seven and eight coordination for first row transition elements. He always attempted to establish the presence and arrangements of complexes of this type by appeal to physical methods while rationalizing their stability and structure in terms of thermodynamic and quantum mechanical theory.

One of Nyholm's real contributions was his insight into the generalities of a subject and his publications have been punctuated by a liberal interjection of review articles in which he attempted to summarize an area with particular emphasis on the generalities underlying the subject. Perhaps the most significant of all these was his Tilden lecture of 1960, in which he reviewed the use of ionization potential-promotion energy data to the understanding of the reactivity and stereochemistry of transition metal complexes and the overall relationship between electron structure and configuration of transition metal complexes³. This work provides us with one of the best summaries of the salient factors in the field. This facility for seeing the general, coupled with his lucidity in speech, made him one of the most popular lecturers on both the national and international scene.

Nyholm's researches in transition metal chemistry ranged over all possible areas but were particularly concerned with metal carbonyl, metal-metal bonded systems, unusual stereochemistries and more recently in the reactivity of coordinated organic groups. He was a prime mover in the development of ligand field theory to inorganic complexes and with Ingold and Tobe initiated mechanistic studies in transition metal complexes. There are few fields of transition metal chemistry in which Nyholm did not make a significant contribution. Recently his expertise in co-

ordination chemistry was extended to the complexes formed by the alkali metals and alkaline earth elements with a variety of polydentate ligands, under the auspices of the ARC. This work emphasized his view that coordination chemistry was an approach to chemistry rather than a branch of it and brought his interests into the bio-inorganic chemical scene. Nyholm's influence in chemistry will continue for many years not only through his published work but also through his students. All who came in contact with him as a research worker were impressed by his attitudes to his chemistry and certainly many will perpetuate the Nyholm philosophy of the subject.

In addition to his research, Nyholm made his impact on virtually all aspects of chemistry. In teaching he was interested in the problems of the school, the technical college and the University. His chairmanship of the Nuffield Schools chemical project was active and his attempts to influence the course of teaching of chemistry realistic. His efforts to transpose difficult theoretical concepts to an understandable form, at the school level, often posed his colleagues with testing questions in their own discipline which plumbed their own understanding. Thus the utilization of the lone-pair repulsion theory in considering the stereochemistry of inorganic molecules, with R. J. Gillespie, allowed for an inclusion of stereochemical consideration into the 'O' level chemistry course, adding a third dimension to school chemistry courses. His influence on ONC and HNC courses of inorganic chemistry in technical colleges was also profound and his recent development into first year university teaching of

chemistry emphasized his progressive attitude to the teaching of the subject. Of the many committees with which Nyholm was associated, his educational interests were of considerable importance to him, both on the national and international level.

His contributions in the areas of industry and government committees were also significant. In particular his active participation in the SRC, both on general committees and as chairman of the chemical committee, allowed his liberal and realistic views to be felt. He was a prime mover in the recent merger of the various Chemical Societies, and during his tenure of the office of President of the Chemical Society he actively pursued the policy to a successful conclusion.

Rightly during his chemical career, Nyholm received many accolades including several honorary degrees and numerous other awards. He was elected a Fellow of the Royal Society in 1958, and was knighted in 1967. He was President of the Chemical Society from 1968 to 1970 and President of the Association for Science Education in 1967.

Ron Nyholm's influence on inorganic chemistry has been deep and he will be sadly missed as a chemist; but many people will miss him primarily as a friend. The students, friends and scientists who have shared in the hospitality of his household will recognize that he was principally a family man, and will wish to extend their sympathy to his family at this time.

¹ *Quart. Rev.*, **3**, 321 (1949).

² *Nature*, **165**, 154 (1950).

³ *Proc. Chem. Soc.*, 273 (1961).

Announcements

Appointments

Professor B. C. L. Weedon has been reappointed chairman of the Food Additives and Contaminants Committee; Professor F. Aylward, Dr J. H. Hamence, Mr J. Saunders and Professor E. F. Williams have been reappointed to the committee, and Dr W. C. Fulton and Professor M. Ingram have been appointed as additional members.

Miscellaneous

The sixth Stephen Rothman award of the Society for Investigative Dermatology Inc. will be presented to Dr William Montagna, director of the Oregon Regional Primate Center.

The Steacie prize for 1971 has been awarded to Professor G. A. Gratzer, University of Manitoba. The award is made annually in commemoration of Dr E. W. R. Steacie, president of the National Research Council of Canada during 1951-62, and consists of a cash award of \$1,500.

The Lomonosov gold medals for 1971—the highest annual award of the Soviet Academy of Sciences, traditionally awarded to one Soviet and one foreign scientist each year—have been awarded to Academician Viktor Amasaspovich Ambartsumyan of the Armenian SSR for his outstanding achievements in astronomy and astrophysics, and to Professor Hannes Alfvén of Sweden for "outstanding achievements in the field of plasma physics and astrophysics".

(Continued from p. 140)

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International Meetings

February 3–5, **Inaugural Meeting, Astronomy Division, European Physical Society**, Meudon (Mme Dreux, DAF Observatoire de Meudon, 92 Meudon, France); February 5, **Business Meeting** to discuss future developments.

February 7–10, **Leisure, Touring and the Environment**, Salzburg (Alliance Internationale de Tourisme, 9 rue Pierre-Fatio, Geneva, Switzerland).

February 10, **Conduction and Magnetic Signalling in the Sea**, London (Information Officer, Institution of Electronic and Radio Engineers, 8–9 Bedford Square, London WC1B 3RG).

February 17, **Noise Abatement Programmes for the Future**, Washington (International Association for Pollution Control, Suite 700, 4733 Bethesda Avenue, Washington DC 20014, USA).

March 6–10, **Analytical Chemistry and Applied Spectroscopy**, Cleveland (Robert W. Baudoux, United States Steel Corporation, Applied Research Laboratory MS 57, Monroeville, Pennsylvania 15146, USA).

March 7–9, **Earth's Core-Mantle Interface**, Melbourne, Florida (American Geophysical Union, 2100 Pennsylvania Avenue NW, Washington DC 20037, USA).

March 22, **Recent Advances in Microprobe Analysis**, London (Dr J. V. P. Long, Department of Mineralogy and Petrology, University of Cambridge, Downing Place, Cambridge CB2 3EW).

March 28, **Beam Detection Techniques**, London (Dr A. R. Burgess, Department of Chemical Engineering, University College London, Torrington Place, London WC1E 7JE).

April 4, **Filtration in Fluid Power**, London (Filtration Society, 1 Katharine Street, Croydon CR9 1LB).

April 4–6, **Computer-Communications Networks and Teletraffic**, New York City (Jerome Fox, Microwave Research Insti-

tute, Polytechnic Institute of Brooklyn, 333 Jay Street, Brooklyn, New York 11201, USA).

April 5–7, **Carbohydrate Chemistry**, Brighton (N. R. Williams, Department of Chemistry, Birkbeck College, Malet Street, London WC1).

April 5–7, **Jet Cutting Technology**, Coventry (Organizing Secretary, First ISJCT, BHRA—Fluid Engineering, Cranfield, Bedfordshire).

April 5–12, **Myriapoda**, Manchester (J. G. Blower, Department of Zoology, University, Manchester M13 9PL).

April 6–7, **Diffusion in Polymeric Systems**, Aberdeen (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

April 6–7, **Cellular Aspects of Ageing**, Norwich (Dr Michael Balls, School of Biological Sciences, University of East Anglia, Norwich NOR 88C).

Erratum

IN the article "Design and Synthesis of Controlled Release Pesticide-Polymer Combinations" by G. G. Allan, C. S. Chopra, A. N. Neogi and R. M. Wilkins (*Nature*, 234, 349; 1971), equation (3) should read:

$$D_{pr} = A - B/\sqrt{W} \text{ from which}$$

$$W = B^2/A^2 \text{ when } D_{pr} = 0$$

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

Cotton Research Corporation 1921 to 1971. (In celebration of the Golden Jubilee of the Cotton Research Corporation, and of the donation of its Cotton Research Station, Namulonge, to the Government of Uganda on 1st January 1972.) Pp. 28. (London: Cotton Research Corporation, 1971.) [2411] A Framework for Government Research and Development. (Cmnd. 4814.) Pp. iii+43. (London: HMSO, 1971.) 524p net. [2611] Central Statistical Office. Social Trends, No. 2, 1971. Edited by Muriel Nissel. (A publication of the Government Statistical Service.) Pp. 205. (London: HMSO, 1971.) £2.90 net. [2611]

Other Countries

Territory of Papua and New Guinea: Department of Agriculture, Stock and Fisheries. Fisheries Bulletin No. 3: Prawn Surveys in Papua and New Guinea. By A. M. Rapson and C. R. McIntosh. Pp. 126. (Port Moresby: Department of Agriculture, Stock and Fisheries, 1971.) [1311] The Test Ban.—SIPRA Research Report. Pp. iv+64. (Stockholm: Stockholm International Peace Research Institute, 1971.) [1511] Organization for Economic Co-operation and Development. Problems of Science Policy. (Seminar held at Jouy-en-Josas, France, 19th–25th February 1967.) Pp. 195. (Paris: OECD; London: HMSO, 1968.) 13 francs; £1.13. [1511] Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel, Exercice 1970. Pp. 315. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1971.) [1511] Stockholm International Peace Research Institute. Report of Activities 1970/71. Pp. 23. (Stockholm: Stockholm International Peace Research Institute, 1971.) [1711] Records of the Australian Museum. Vol. 28, No. 7 (4 October 1971): Balls Head: The Excavation of a Port Jackson Rock Shelter. By Sandra Bowdler. Pp. 117–128+plates 17–21. (Sydney: The Australian National Museum, 1971.) 80c. [1711] Mauritius Sugar Industry Research Institute. 18th Annual Report 1970. Pp. 185+xxxiii. (Reduit: Mauritius Sugar Industry Research Institute, 1971.) [1711] Records of the Australian Museum. Vol. 28, No. 6: A New Species of *Kelleria* (Copepoda: Cyclopoida) from Brackish Water in Victoria. By I. A. E. Bayly. Pp. 111–116. (Sydney: Australian Museum, 1971.) 30c. [1811] Transvaal Nature Conservation Division—Fifth Annual Report, 1969/1970. Pp. 39. (Pretoria: Transvaal Provincial Administration, 1971.) [1811] Commonwealth of Australia. Nineteenth Annual Report of the Australian Atomic Energy Commission for the year ended 30 June 1971. Pp. 147. (Coogee, NSW: Australian Atomic Energy Commission, 1971.) [1811] Museum of Applied Arts and Sciences, Sydney. Annual Report of the Trustees, 1970. Pp. 23. (Sydney: Museum of Applied Art and Sciences, 1971.) [1811] Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Memoir 365: Geology of Beechey Lake Map-Area, District of Mackenzie, a Part of the Western Canadian Precambrian Shield. By L. P. Tremblay. Pp. 56. \$2.50. Paper 70–68: A Subdivision of the Upper Cretaceous–Lower Tertiary Sustut Group, Toadoggonne Map-Area, British Columbia. By G. H. Eisbacher. Pp. v+16. \$1.50. Paper 71–43: Mercury in Permafrost Regions: Occurrence and Distribution in the Kaminak Lake Area, Northwest Territories. By E. H. W. Hornbrook and I. R. Jonasson. Pp. 13. \$1.50. (Ottawa: Information Canada, 1971.) [1911] Australia: Commonwealth Scientific and Industrial Research Organization. Applied Geomechanics Research Report 1971. Pp. 52. (Mount Waverley, Victoria: Division of Applied Geomechanics, P.O. Box 54, 1971.) [1911] Veröffentlichungen der Bibliothek der Bergakademie Freiberg. Nummer 40: Zur Geschichte der Geologie, Geophysik, Mineralogie und Paläontologie, Freiberg 1970. Von Peter Schmidt. Pp. 134. (Freiberg: Bergakademie, 1970.) [2211] World Meteorological Organization. Technical Note No. 116: Investigations on the Climatic Conditions of the Advancement of the Tunisian Sahara. By H. Flohn. Pp. 28. (Geneva: World Meteorological Organization, 1971.) [2211] World Meteorological Organization. Guide on the Global Data-Processing System. Vol. 2: Preparation of Synoptic Weather Charts and Diagrams. Pp. 68. (Geneva: World Meteorological Organization, 1971.) [2211]

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